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Investigating the role of microRNAs, inflammation, and *Helicobacter pylori* in Epstein-Barr virus associated gastric cancer

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Abstract

Epstein-Barr virus-associated gastric carcinoma (EBVaGC) is a distinct subtype that accounts for nearly 10% of gastric carcinomas. This type of gastric cancer has no relation to any mutation in chromosomal genes, and EBV causes cancer by affecting the epigenetics of host cells through methylation and inactivation of the promoter of tumor suppressor genes. This suggests that EBV infection precedes the clonal growth of EBV-infected cells and subsequently develops carcinoma. Chronic gastritis in the background of EBVaGC might enhance the chance of interaction between gastric epithelial cells and B lymphocytes, and cytokines produced by inflammatory cells might support the growth of EBV-infected gastric epithelial cells. Numerous modifiable risk factors have been identified for gastric cancer (GC). Inflammation is a complicated host immune response to biological, chemical, and physical invasions. Chronic inflammation, which is caused by genetic mutations, autoimmune diseases, constant exposure to environmental factors, and viral infections, can significantly increase the risk of cancer. According to epidemiologic studies, chronic infection and inflammation are considered the main risk factors for different types of cancer. Furthermore, although oncogenic viruses stimulate inflammation by dint of different mechanisms, they generally activate certain signaling pathways, including NF-κB and STAT3, in charge of cancer development. The role of EBV in chronic gastric inflammation has received little attention. However, several studies have indicated that EBV as well as *Helicobacter pylori* is initially involved in the oncogenic process of GC by increasing chronic inflammation and tissue damage. Moreover, other risk factors, including lifestyle and HPV infection, play a role in the progression of GC.

Keywords: Gastric cancer, Epstein Barr virus, *Helicobacter pylori*, inflammation, microRNA

1. Introduction

Gastric cancer (GC) has been indicated as one of the cancers with a high mortal rate particularly among older males. GLOBOCAN 2018 data reports GC as the 5th most common cancer and the 3rd leading cause of cancer-related mortality; and estimates 783,000 deaths in 2018 (1). GC is one of the most often diagnosed malignancies globally, with a highly dismal prognosis (2). Considering the type of cell involved, GC is classified into the following 4 types: 1) Adenocarcinoma: in the gastric inner lining cells (mucous surface); 2) Lymphoma: immune system cancer in the gastric lymphoid tissue, which is extremely rare; 3) Gastrointestinal stromal tumors (GIST): the gastrointestinal epithelial tumors in the interstitial Cajal cells, which occur rarely; 4) Carcinoid tumors are generally formed in the cells secreting the gastrointestinal hormone (3). Over 90% of gastric tumors are adenocarcinomas, and etiological factors such as socioeconomic conditions, diet, hereditary factors, *Helicobacter pylori* (*H. pylori*) infections, and the Epstein Barr Virus (EBV) are significantly reported to be involved in their pathogenesis (4, 5). GC manifests in various symptoms; however, patients are often diagnosed in the advanced stages. Hence, the identification of the risk factors along with their early treatment can be helpful in the prevention of GC development (6).

EBV infection is related to certain types of cancer, including Hodgkin's lymphoma (HL), Burkitt's lymphoma (BL), lymphoma in diarrhea patients, and some other carcinomas. This infection was first discovered in 1964 by Anthony Epstein and Yvonne Barr, who used electron microscopy to identify the herpes simplex virus in a subpopulation of BL cells in African patients (7, 8). EBV is a herpes virus that infects more than 90% of the world population

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before adolescence. This virus has been observed in epithelial malignancies including GC (9). EBV-associated gastric carcinoma (EBVaGC) accounts for 8 to 10% of cases, and it is estimated to infect more than 90,000 individuals yearly (10). Following the onset of the infection, EBV remains latent in the B-lymphocytes at a rate of 1/106. EBV is hard to diagnose since the expression of very few numbers of viral proteins provides the preservation, control, and proliferation of the cells (11, 12). In a pediatric study, infection with EBV was reported to be the main cause of severe gastritis and chronic inflammation in comparison to the separate infections of the related pathogen (13).

Furthermore, H. pylori infects about 50% of people worldwide which induces gastric inflammation and optimizes the necessities for EBV tumorigenesis. It has been demonstrated that overexpression of inflammatory markers and epigenetic alterations such as hypermethylation associated with EBVaGC are due to H. pylori infection (14).

Inflammation is defined by the attempt of the immune system to fight against infections, injuries, and toxins, and is characterized by the infiltration of mononuclear cells, especially macrophages in the damaged tissue. Due to the presence of inflammatory cells in tumor tissues, it was suggested that chronic inflammation may play a key role during carcinogenesis through persistent activation, leading to continuous tissue damage. Later, it was determined that about 25% of all cancer types including GC are associated with chronic inflammation. H. pylori causes chronic gastritis and there is a well-known correlation between H. pylori and chronic inflammation, which together result in gastric adenocarcinoma (15, 16).

Randomized clinical studies show that individuals with gastroesophageal adenocarcinoma and nonmetastatic GC benefit from combined treatment. Although postoperative chemotherapy following an appropriate lymph node dissection is a treatment choice, current recommendations identify perioperative chemotherapy or postoperative chemotherapy with chemoradiation as preferable treatments (17).

MicroRNAs (miRNAs) are a class of non-coding RNAs (ncRNAs) that modulate gene expression by suppressing translation. It is noteworthy that the expression of these RNA molecules can be regulated by further DNA methylation and chemical modifications of the histones. It has been extensively reported that mutations or the wrong expressions of miRNAs are associated with various human cancers, indicating their ability to inhibit tumorigenic and oncogenic agents (18, 19). EBV can encode miRNAs in its own DNA sequences. Various genomic profiling studies have claimed EBV-encoded miRNAs play a crucial role in EBVaGC (20).

In the current study, we reviewed the role of chronic inflammation, H. pylori infection, HPV infection, viral miRNAs and lifestyle during the development and progression of EBVaGC (Fig.1).

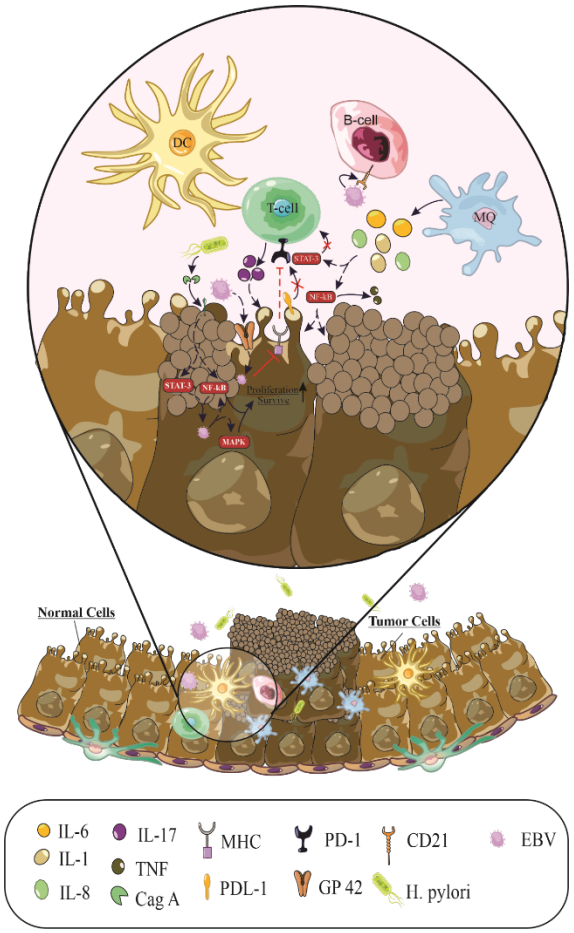

Fig. 1. Co-infection of H. pylori infection and EBV

Infection with *H. pylori* leads to the activation of a series of intracellular signaling pathways, followed by the reactivation of latent EBV infection. Oncogenes expressed by EBV cause disturbances in the identification of tumor cells and subsequently reduce the response of immune cells during infection and thus increase the development of malignancy.

2. Chronic Infection-related inflammation, and carcinogenesis

It has been reported that chronic inflammation is associated with tumorigenesis as well as increased cell proliferation, cell survival, invasion, angiogenesis, and metastasis (21). According to the estimates, infectious diseases and chronic inflammation make up approximately 25% of carcinogens (22). Research findings also indicate that inflammation suppresses the DNA repair system (23). On the other hand, macrophages (MQ) play a key role in chronic inflammation and can trigger a pro-inflammatory response by secreting inflammatory factors and several prototypical pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8 (24, 25). In this regard, interleukin-10 (IL-10) is an important anti-inflammatory cytokine capable of suppressing DNA damage (26). Persistent infection can result in an over-inflammatory response that is generally harmful to the host by exerting mutagenic effects on the host genome (27). The onset of cancer in the course of a viral infection is classified into two categories

considering the capacity of virus for direct or indirect involvement in the stimulation of cell proliferation and/or interfere with apoptosis, playing a direct role in carcinogenesis (28). Inflammatory excitation and the direct effects of the virus also activate the signaling pathways responsible for carcinogenesis including Nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription3 (STAT3) (29, 30).

NF-κB and STAT3 are two major factors linking inflammation to cancer. NF-κB is considered as pro-inflammatory signaling pathway due to its activation by proinflammatory cytokines and tumor necrosis factor α (TNFα). NF-κB controls several cellular processes including proliferation, apoptosis, and angiogenesis. The NF-κB signaling pathway is considered as an apparent mediator between inflammation and development of various cell populations in the stomach to cancer. Activation of NF-κB is crucial for regulating immune responses. It has been reported that aberrant expression of NF-κB can lead to inflammation and therefore cancer-associated inflammation, especially in human gastrointestinal cancer (29, 31).

On the other hand, the STAT family proteins, especially STAT3, play crucial roles in the induction of a pro-carcinogenic inflammatory microenvironment. STAT3 can be activated by various genes, including cytokines and oncogenes. It has been reported that continuous activation of STAT3 suppresses anti-cancer immunity and therefore induces tumor cell survival proliferation, and invasion. Recently, what has been investigated? GC? has been investigated the role of NF-κB and STAT3 in a few cancer types including GC. Also, the activation and interaction between STAT3 and NF-κB play a key role in the connection between cancer cells and inflammatory cells (29, 32).

In chronic inflammations, ROS/RNS are released by inflammatory cells as well as the epithelial cells, stimulating DNA damage in the affected organs and instigating the risk of carcinogenesis (33, 34). Epigenetic gene silencing also significantly contributes to carcinogenesis by reducing the expression of the tumor suppressor genes and microRNAs. In inflammatory microenvironments, exposure to ROS/RNS or pro-inflammatory cytokines such as IL-6 influences the expression of DNA methyltransferase 1 (*DNMT1)*, increasing DNA methylation in the tumor suppressor genes and miRNAs (35). Malignant human tumors are characterized by the alterations in the DNA methylation patterns. This process involves the general hypomethylation of cancer cells and hypermethylation of some CpG islands, most of which are within gene promoters (36). DNA methylation in GC rich regions is an important stimulus for the oncogenic process and is linked to *H. pylori* and EBV infections (37, 38).

3. The Role of EBV in gastric carcinogenesis

It was initially proposed that EBV infects B cells only, later it was reported in the nasopharynx epithelial cells (39), liver cells (40), gastric epithelial cells (41), and brain cells (42). According to the previous studies, EBV may be transmitted into the cells through the oral epithelium by the EBV+IgA combination (43). The EBV transmission potentially results in the early penetration of the virus into the B cells, triggering a systemic infection. In patients infected with EBV, the virus can also be secreted in the saliva (44, 45). The B-cell surface receptor for EBV is CR2, or CD21. The EBV envelope glycoprotein, gp350/220, is a ligand that binds to the cell's CD21 surface marker (46). The second EBV receptor on the B-lymphocytes is the class II HLA molecules, where the virus binds them through the GP42 glycoprotein. The class II HLA molecules are not present on the epithelial cells, and thus it can be proposed that the glycoprotein GP42 is not necessary for the EBV infection of epithelial cells. Nevertheless, not only is GP42 not regulated in epithelial cell infections, but its presence can also impose inhibitory effects (47, 48). Herpes viruses are suggested to have two different life cycles: lytic replication and latent replication. EBV can either lead to latent infection or enter a lytic cycle in the host cells; however, expressing some viral genes, including ncRNAs, prefers to remain latent. The phase change from latent infection to the lytic cycle is done through two genes, BZLF*1* and BRLF*1,* which encode the two transcription factors Zta and Rta, respectively (49). In particular, EBV can infect the host gastric epithelial cells by employing direct and indirect mechanisms. In direct infection, viral glycoproteins bind to the cell receptors, modifying the viral proteins with constructive changes and culminating in the consequent increase in the fusion of viral envelopes and epithelial cellular membranes (50). EBV is most probably not a passive carrier but rather the oncogene of an active virus contributing to the development of GC in the early stages (51). In recent years, it has been increasingly reported that EBV may contribute to GC through the expression of viral proteins and miRNAs as well as by inducing aberrant DNA methylation in CpG islands and posttranslational histone modification (52). These alterations are suggested by the existing scientific evidence to be involved in the development of EBVaGC (53).

4. EBV gene expression in infected cells and their functions

Considering the subset of the expressed viral genes, the herpes virus-related tumors are classified into the following four categories: latency Ia, latency Ib, latency II, and latency III. EBVaGC belongs to the latency type I category, which includes Epstein-Barr virus latent membrane protein 2A (LMP2A), Bam-HI A rightward transcripts (BARTs), and Epstein-Barr virus (EBV)-encoded EBV-encoded small ribonucleic acid 1/2 (EBER1/2) (54, 55). LMP1 and LMP2 activate the well-known NF-κB and MAP kinase signaling pathways, which are responsible for cell survival and proliferation (56, 57). The viral LMP2A protein also influences the NF-κB pathway and increases the production of survival genes. Therefore, it increases apoptosis inhibition as well as cancer stem cells induction in the EBV-associated epithelial cancers (58). Several cases of the involvement of BamHI-A

rightward frame 1 (*BARF-1*) in GC have also been reported. Almost every case of EBVaGC has the BARF-1 gene expressed (59). BARF-1 is demonstrated to enhance cell proliferation by regulating the NF-κB and cyclin-D1 expressions in EBV-infected gastric carcinoma cells. Besides, BARF-1 reduces expression by inhibiting p21 (60). EBNA1 is an EBV-encoded sequence-specific DNA-binding protein that is consistently expressed in EBV-associated tumors and required for stable maintenance of the viral genome in proliferating cells. EBNA1 is also thought to play a role in cell survival in latently infected cells (61). Inhibition of EBNA1 through compound inhibitors diminished the particular EBV genome copy number in Raji Burkitt lymphoma cells (62). EBERs are the most abundant genes among the latent ones in the infected cell (63). So, EBER1-*in situ* hybridization (ISH) is considered as the gold standard method to detect EBV. There are some findings around the role of EBER1 in the EBVaGC. As it is obvious, downregulation of E-cadherin is necessary for tumorgenicity. It has been reported that EBER-1 can dysregulate the cellular miRNA expression levels to suppress E-cadherin, and therefore induce epithelial-to-mesenchymal transition (EMT) in gastric carcinoma cells (64). Interestingly, Banerjee *et al.* proved that EBERs can upregulate pro-metastatic markers such as pFAK and pPAK1, and suppress anti-metastatic factors, which accounts for cell migration. Further, EBERs could promote chemoresistance by indirect downregulation of the p21 and p27 cell cycle inhibitors (65).

4.1. Immune responses in EBVaGC

During the infection of epithelial cells, the EBV encoded regulatory viral RNAs might modulate the host's innate immune responses (66). Moreover, BARF1, BART miRNA, EBER1/2, and EBNA-1 inhibit the interferon response. EBNA-1 and BNLF2 interfere with the antigen presentation by MHC molecules and the identification via CTL. Tumor cells also express the programmed death-ligand 1 (PD-L1), which binds to the PD-1 receptor in CTLs and other immune cells and inhibits the effective immune response. Similarly, BART miRNA inhibits the expression of major histocompatibility complex class 1-related chain B (MICB) to prevent NK cell and CTL recognition (67, 68). The different forms of CD44, which is a cell surface glycoprotein and functions as an adhesion molecule, are especially expressed in EBVaGC (69). EBVaGC lymphocytes are primarily CD8-positive cytotoxic T cells (70), which improve antitumor immunity (71). However, during tumor growth, the exact mechanism of the carcinoma cells preventing the host immune response is not fully understood. In this regard, IL-1β is the only cytokine that is considerably expressed on a large scale in EBVaGCs as compared to EBVnGCs. IL-1β may use a large number of nonspecific lymphocytes to protect EBV-specific cytotoxic T cells and tumor cells from direct exposure (72, 73). It also inhibits the secretion of gastric acid, allowing for the growth of EBVaGC (74). Infiltrating protective cells at a minimum degree aids antitumor defenses by increasing the complete destruction of EBV-positive cancerous cells (70, 71, 75). Finally, the expression of EBER1/2 and BART miRNAs improves the immune suppressor level, i.e. IL-10 expression (76, 77).

4.2. The Role of MicroRNAs in EBV-Associated Gastric Carcinogenesis

It has been highlighted in EBVaGCs that microRNAs, as well as long noncoding RNAs (lncRNAs), play significant roles in the regulation of gene expression following transcription (78, 79). This process involves approximately 25 viral miRNA precursors and 44 mature microRNAs, which are classified into two large clusters: miR-BART and miR-BARF-1. The targetome of EBV miRNAs are associated with signal transduction, oncogenesis, cell adhesion, and apoptosis, all of which contribute essentially to carcinogenesis (80). For instance, BART reduces BID (BH3 interacting-domain death agonist) expression, which is an apoptotic molecule. Furthermore, miR-BARTs are more abundant in NPC and EBVaGC than in EBV-positive B lymphoma (79, 81). It was also found that miR-BART20-5p suppressed lytic replication by directly targeting *BRLF1* and *BZLF1* (49). Further, it improves the discrimination of the apoptosis factor BAD (BCL2 associated agonist of cell death) and stimulates the proliferation of GC cells through BAD suppression (82). Besides, it has been reported that miR-BART20-5p could target BID, which belongs to the Bc1-2 gene family and suppresses cell death in GC cells. Regarding the targeting of BAD and BID, it was indicated that miR-BARTs suppress cell death by targeting different genes (80, 83). When EBVaGC is induced by latent EBV infection, it expresses low amounts of viral antigen to enable the virus to escape the immune system, maintaining a certain degree of infection. MiRBART6 also stimulates latent EBV infections. The host cellular miRNAs can be dysregulated by the latent EBV genes, resulting in epithelial-mesenchymal transition (EMT). Moreover, the levels of two host miRNAs, miR-200a and miR-200b, are decreased in EBVaGC. MiR-200a and miR-200b target two transcription repressors, ZEB1 and ZEB2, which control E-cadherin expression levels. Therefore, downregulation of the aforementioned miRNAs can lead to EMT and promote tumorigenesis. The EBV-derived miRNAs prevent the translation of viral and host mRNAs. For instance, Shinozaki *et al.* reported that EBV-encoded LMP2A suppresses the expression of pri-miR-200 as a receptor of EMT (64, 79, 80, 84). These reports also suggest that EBV infections change the attributes of the host cells, which may increase the metastatic activity of tumor cells infected with EBV.

4.3. *H. pylori* in GC

Marshall and Warren earned the Nobel Prize for Medicine in 2005 for proving that *H. pylori* contributes to peptic ulcer disease (85). *H. pylori* is a spiral-shaped, gram-negative, urease-positive bacteria (86) that resides in the human gastric and duodenum, which is present in the body of half of the world's population. Peptic ulcers, GC, MALT lymphoma, and

other extra-gastrointestinal disorders have all been linked to *H. pylori* as a cause (87, 88). *H. pylori* has been categorized as a Group 1 carcinogenic pathogen, according to the International Agency for Research on Cancer (IARC). In both Western and Eastern nations, *H. pylori* infection is regarded as a significant risk factor for GC (89).

H. pylori encodes a wide range of genes that are involved in its pathogenicity and microenvironment modification including urease, carbonic anhydrase, Lewis antigen, VecA, CagA and outer proteins (BabA2) (90). The virulence factors produced by *H. pylori* can alter the signaling pathways in the host cell. *H. pylori*'s ability to survive for decades in the stomach environment due to the host's inability to eradicate the infection makes this trait particularly significant. Due to the pathogen's urease, which converts urea to ammonia and creates a neutral environment around the bacteria, it may colonize the stomach's extremely acidic environment. *H. pylori* is capable of eluding host immune responses while interacting with gastric cells and surviving in the severe environment of the gastric corpus (85). In order to create an environment that is immunosuppressive and supports chronic infection, *H. pylori* aggressively manipulates host tissues. *H. pylori* inhibits the effector activities of CD4+ T cells, dendritic cells, and macrophages while promoting the production of regulatory T cells and myeloid-derived suppressor cells (91). In conclusion, Lewis antigen is a crucial protein that supports *H. pylori* survival in difficult habitat circumstances, followed by CagA, Bab, and VacA that cause *H. pylori* to inhabit the gastric epithelium and cause inflammation. The evolution of *H. pylori* is being served by all of these genes and the proteins that are produced as a result of them (90).

5. EBV co-infection with *H. pylori* in GC

Several reports have revealed the synergistic effects of *H. pylori* and EBV in the development of GC. In the initial phases, patients suffering from *H. pylori* and EBV demonstrate severe inflammatory signs compared to patients suffering from *H. pylori* alone (13). Another study on co-infections suggests that EBV accompanied by *H. pylori* induces inflammatory responses in patients, increasing the risk of gastrointestinal cancer progression (92). It was also indicated that *H. pylori* infection is associated with EBV reactivation in patients showing gastric signs (93). Moreover, the activation of EBV in the latent cycle of the infected gastric epithelial cells is incited by monochloramine, which is produced by *H. pylori* (94). According to this evidence, the co-infection of these pathogens can probably increase the risk of GC (95, 96). Furthermore, in the *H. pylori*-positive patients, the level of EBV DNA is often evidently higher, suggesting the role of *H. pylori* in the transformation of the lytic EBV cycle (94). Two possible mechanisms are involved in this process. The first mechanism induces an additional inflammatory response in the co-infection, where both the EBV and *H. pylori* can increase tissue damage (13, 97). Therefore, a significant increase in IL-1β (98), TNFα (99), and IL-8 (100), levels is observed. The second mechanism involves the interactions among the gene products, which mainly happen between EBV and *H. pylori*. The findings from an in vitro study indicated that the EBV reaction takes place via the PLC_{γ} signaling pathway, while an *H. pylori* toxin known as CagA severely activates PLC_{γ}, as well as several other kinases (101, 102). CagA in *H. pylori* and LMP1 and LMP2 in EBV activate the MAP (mitogen-activated protein) kinase and NF-κB pathways, which are the well-known pathways for the proliferation and survival of the cells during carcinogenesis (56, 57). *H. pylori*, using CagA oncoprotein, triggers the unusual activation of the WNT signaling pathway, resulting in the activation of CDX1 as a downstream gene (103, 104). Both pathogens share several pathways and activate the transformer factors in the gastric epithelial cells via the β-catenin signaling pathway (13, 105). Some studies have also indicated that *H. pylori* reduces the expression of TGF-β, which reactivates the lytic phase of EBV. Hence, it may be involved in the prevention of the reactivation of the lytic phase of EBV and prevention of GC (106). Therefore, more studies must be carried out on the co-infection of *H. pylori* and EBV to unveil the potential roles of both pathogens.

6. HPV infection and GC

Another factor associated with GC is human papilloma virus (HPV) infection (107). HPVs are part of the family of DNA viruses named Papillomaviridiae family, and new species are continually being identified. The epithelia of the upper respiratory tract, genitalia, and skin are where this virus exhibits the highest rate of tropism (108-110). According to findings, HPV is one of the key infectious agents contributing to prostate, cervical, anal, and colorectal cancer as well. The majority of studies contend that co-occurring HPV and *H. pylori* can cause cancer, while some research indicates no such association (111-114). According to studies, high-risk HPV types 16 and 18 are closely associated with GC specimens, which may serve as a warning to those in the control group who tested positive for these HPV subtypes (107, 115). Moreover, immunization against this virus (HPV) has to be pursued more aggressively in order to hinder malignancies linked to it (107). HPV is thought to increase the chance of developing neoplasms. Neoplastic transformation is typically a protracted, extremely complicated, and multi-step process and is brought on by numerous genetic and epigenetic variations (116). However, there is some debate on the connection between GC and HPV infection and more study is required to be sure (108).

7. Epigenetic modifications in EBVaGC: DNA methylation

Several aberrantly methylated genes have been observed in gastric adenocarcinomas caused by EBV and *H. pylori* co-infection. The most common hypermethylated genes are *CDK2A*, *CDH1*, *DAPK*, *COX2*, and *MLH1*. These genes are often altered in different cancers including GC (81). Methylation of tumor suppressor genes is the main cause of the unusual state of EBVaGC. In EBVaGC tumor cells, methylation of different CpG regions in the tumor-associated

promoters has been repeatedly observed and substantial roles have been attributed to them in the development of GC (117, 118). CpG island methylation is an epigenetic process in gene presentation, influencing all cellular pathways (119). Higher than half of genes hold the CpG site in the promoter section which is found as CpG islands (120). Methylation in CpG sites inside of a promoter region could suppress binding in transcription conditions to the succession of neoplasm suppressor genes (121). The repeated methylation of the tumor suppressor genes (such as *RASSF1A*, *PTEN*, and *APC*) and the adhesion genes (e.g., *THBS1* and E-cadherin) in EBVaGC are evidently higher as compared to EBV-negative samples (122, 123). Moreover, the EBV infection is associated with an increase in the expression of *DNMT1* in gastric carcinomas (124). The high frequency of methylation in the genome has been reported in EBVaGC. Besides, several methylated genes including *RUNX3, p73, p16, DAPK1, PTEN, RASSF1A*, and *GSTP1* are often observed in EBVaGC as well as EBV-negative GC although with lower methylation levels (125, 126). EBV-related LMP2A activates several cellular signaling pathways including the PI3K/AKT and JAK/STAT3 that carry out most *DNMT* regulations as well as other epigenetic modifiers during the EBVaGC pathogenesis. LMP2A can increase *DNMT3b, DNMT1*, and the expression of B lymphoma Mo-MLV insertion region 1 homolog (*BMI1)* on the transcription and translation levels. Besides, it increases the expression of *DNMT1* by inducing STAT3 phosphorylation, which is independent of the stimulation of IL-6. It increases PTEN methylation, suppressing it in EBVaGC (127, 128). CpG island methylation in the promoter regions (CIMP) of the tumor suppressor genes such as *PTEN* and *CDKN2A* also occurs. This process entails the structural activation of the PI3K pathway and the inactivation of the cell cycle checkpoints. The Akt, PI3K, and mTOR inhibitors, beta-catenin, and notch can also act against the EMT, and stemming resistance mechanisms (129, 130). On the other hand, LMP1 is rarely expressed and this protein is not generally expressed in EBV-associated gastric carcinoma. The transfer of BARF1 to a GC cell makes considerable changes to the host gene expression. It particularly changes the expression of genes associated with proliferation and apoptosis. BARF1-transfected cells demonstrate chemical resistance and higher expression of Bcl-2 in comparison to Bax (131, 132). The various expression patterns connected to the three stages of the life cycle of the EBV are controlled by epigenetic principles (133). All in all, the methylation of both viral and host DNA strands is one of the major mechanisms involved in the development of EBVaGC. The EBV infection of epithelial cells can result in DNA methylation. Viral DNA methylation prevents recessive EBV genes. Finally, the DNA methylation of the host cells inactivates the tumor suppressor genes and the associated antigens (134).

8. Lifestyle

Primary cancer prevention through lifestyle and dietary modifications is still a top focus since it is an essential technique for lowering the population burden of many cancers. Physical exercise and lifestyle variables like relative body mass are thought to be major modifiable factors in cancer prevention (2). Several dietary exposures have been related to GC; however, the connections may be influenced by intrinsic biases. Tobacco usage, alcohol drinking, salt-preserved foods, age, gender, medical history and industrial and chemical pollutants are also known to be connected with an elevated GC risk (135, 136). Tobacco smoking has been linked to 11% of GC cases globally. Similarly, alcohol use has been linked to the development of GC. A big pooled investigation discovered a link between high alcohol use and the chance of developing GC (137). Moreover, fruit consumption may be beneficial to both genders (137). Certain risk factors include *H. pylori* infection as well (137).

As estimated, half of the people worldwide are thought to have *H. pylori* infection though its frequency varies geographically (89). Improvements in living conditions have led to a decline in *H. pylori* infection rates in some nations, although the prevalence is still high in the majority of underdeveloped nations (89), that's the reason why infections with *H. pylori* are less common in developed countries than in developing ones (87). Depending on socioeconomic condition and the quality of hygiene, the prevalence of *H. pylori* infection has been estimated to range from 41.5% to 72.3% in China (89).

9. Other factors influencing the progress of GC

There are other factors influencing the progress of GC P53 mutation and overexpression are common during the development of GC and are therefore recognized in cancer areas as well as precancerous dysplasia and metaplasia (138). This approach points out of that p53 mutation may be an earlier occurrence for GC. EBVaGC usually shows more varieties of p53 in contrast to EBV-negative GC (139). Mainly because GC malignancy is connected to *H. pylori*, some sort of influencing factor with severe gastritis, abdominal metaplasia, and cancer malignancy located primarily on the antrum. These kinds of pathogenic agents happen to be a reason for influencing GC malignancy with an independent process (140). In addition, regular prognosis with both EBV and *H. pylori* in the mucous membrane having reasonable for chronic atrophic gastritis with instigative cell infiltration (95).

Factors affecting the development of GC are summarized in Table1.

Table1. Factors affecting the development of gastric cancer

Factor	Component	Function/Mechanism	References
Inflammation and immune responses	Macrophages	Triggering a pro-inflammatory response by secreting inflammatory factors and several prototypical pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8	(24, 25)
	Over-inflammatory responses	Mutagenic effects on the host genome	(27)
		Suppressing DNA repair system	(23)
	NF-κB	Activated by proinflammatory cytokines and tumor necrosis factor α (TNFα)	(29, 31)
		Controlling several cellular processes including proliferation, apoptosis, and angiogenesis	
		Mediator between inflammation and development of various cell populations	
	STAT3	Activated by various genes including cytokines and oncogenes	(29, 32)
		Induction of a pro carcinogenic inflammatory microenvironment	
		Continuous activation of STAT3 suppresses anti-cancer immunity and therefore induces tumor cell survival proliferation, and invasion	
	ROS/RNS	Stimulating DNA damages	(33, 34)
	PD-L1	Binding to the PD-1 receptor in CTLs and other immune cells and inhibiting the effective immune response	(67, 68)
	IL-1β	Inhibiting the secretion of gastric acid, allowing for the growth of EBVaGC	(74)
Epigenetic gene silencing	MicroRNAs	Reducing the expression of the tumor suppressor genes and microRNAs	(35)
	DNA methylation	Influencing the expression of *DNMT1*, increasing DNA methylation in the tumor suppressor genes and miRNAs	
		DNA methylation in GC rich regions is a stimulus for the oncogenic process	(37, 38)
		Viral DNA methylation prevents recessive EBV genes. Finally, the DNA methylation of the host cells inactivates the tumor suppressor genes and the associated antigens	(134)
Epstein-Barr virus	BZLF*1* gene and BRLF*1* gene	Encoding two transcription factors named Zta and Rta	(49)
	Virus itself	Inducing aberrant DNA methylation in CpG islands	(52)
		Posttranslational histone modification	
	LMP1 and LMP2	Activating NF-κB and MAP kinase signaling pathways, which are responsible for cell survival and proliferation	(56, 57)
	BARF-1	Enhancing cell proliferation by regulating NF-κB and cyclin-D1 expressions in the EBV-infected gastric carcinoma cells	(60)
		Reducing cell expression by inhibiting p21	
		Inhibiting interferon response	(67, 68)
	EBNA1	Causing stable maintenance of viral genome in proliferating cells	(61)
		Providing cell survival function in latently infected cells	
		Interfering with the antigen presentation by MHC molecules and the identification via CTL	(67, 68)
	EBER1	Dysregulating the cellular miRNA expression levels to suppress E-cadherin (which is necessary for tumorgenicity) and therefore inducing epithelial-to-mesenchymal transition	(64)
		Upregulating pro-metastatic markers such as pFAK and pPAK1	(65)
		Suppressing anti-metastatic factors	
		Promoting chemoresistance by indirectly downregulation of the p21 and p27 cell cycle inhibitors	
		Inhibiting interferon response	(67, 68)
		Up regulation of interleukin-10	(77)
MicroRNAs	miR-BART (miR-BART20-5p)	Suppressing lytic replication by directly targeting *BRLF1* and *BZLF1*	(49)
		Improving the discrimination of the apoptosis factor BAD	(82)
		Stimulating the proliferation of gastric cancer cells through BAD suppression	
	miR-BARF-1	Having a role in gastric cancer development	(82)

Table1. Factors affecting the development of gastric cancer (continue)

Factor	Component	Function/Mechanism	References
Other infections	H. pylori	Producing monochloramine by *H. pylori* and cause EBV reactivation	(94)
		Having a role in the transformation of the lytic EBV cycle	
		Inducing an additional inflammatory response and increasing tissue damage	(13, 97)
		Interactions among the gene products e.g. EBV reaction takes place via the PLC_{γ} signaling pathway, while an *H. pylori* toxin known as CagA severely activates PLC_{γ},	(101, 102)
		Activating the transformer factors in the gastric epithelial cells via β-catenin signaling pathway	(13, 105)
	HPV	High-risk HPV types 16 and 18 are closely associated with stomach cancer specimens	(107)
Oncogenes	P53	P53 mutation is recognized in cancer areas and additionally for sectors of precancerous dysplasia together with metaplasia	(138)
Lifestyle	Smoking	Known as a risk factor for gastric cancer	(135, 136, 141)
	Body mass		
	Alcohol drinking		
	Diet		

10. Treatment of EBVaGC

Several studies have determined the resistance of EBVaGC to some chemotherapy drugs, including docetaxel and 5-FU. Recently, some researches have shifted forward to test the clinical response to anti-PD1 inhibitors in EBVaGC (14). It has been reported that PD-L1 is overexpressed in EBVaGC. Sho Sasaki1 *et al*. found that in EBVaGC cell lines with highly expressed PD-L1, the proliferation of T-cells was suppressed by PD-L1 overexpression. Further, using PD-L1 antibody treatment led to a moderately lost G0/G1 arrest of the T-cells in EBVaGC (142). In another study, it was determined that in GC patients who are resistant to chemotherapy, the anti-PD-1 antibody nivolumab has a better prognosis and prolonged survival relative to conventional chemotherapy (143).

On the other hand, as hypermethylation is considered as one of the mechanisms underlying EBVaGC, some preclinical studies showed that demethylating agents such as 5-Aza cytidine could be considered as a potential treatment by restoring the expression pattern of methylated genes and stimulating lytic infection, which results in cell lysis (144).

Another approach is using small-molecule EBNA1 inhibitors. EBNA1 plays a key role in EBV-associated cancers. It was determined that applying EBNA1 inhibitor treatment in Raji cells decreases the EVB copy number in a dose-dependent manner in affected cells (145).

The development of EBV vaccines is another strategy for preventive and also clinical use. The particular gp350 glycoprotein is actually for the majority of vaccines being used as an antigen. Moreover, EBNA1, as well as LMP2A, are also utilized for antigens (146).

11. Conclusion

Worldwide, about 90% of people are infected with EBV before adolescence. We reviewed the highlighted risk factors in developing EBVaGC. Chronic inflammation is an important risk factor for EBVaGC which develops tumorigenesis. Infectious disease along with chronic inflammation results in about 25% of malignancies. The findings from several studies suggest that H. pylori helps EBV remain in the latent phase. Infection with EBV also changes the miRNA-related activities of the host cells, and these modifications may increase the metastatic activity of the EBV-infected tumor cells. It triggers the considerable LMP2-induced methylation of the host genome, leading to hypermethylation of several unique methylated genes in EBVaGC. Furthermore, several cellular pathways in EBVaGC are dysregulated, contributing to tumorigenesis. These pathways improve the proliferation. Many modifiable risk factors have been identified for GC including lifestyle, HPV infection, H. pylori infection, and immune response and DNA methylation. Recently, advances in the PD-L1 inhibitors approach makes better prognosis in GC patients. Taken tougher, a better understanding of the molecular mechanisms of EBVaGC may account for finding novel treatment approaches for GC patients.

Conflict of interest

None to declare.

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Authors' contributions

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