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Tumour Review

Outlooks on Epstein-Barr virus associated gastric cancer

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ABSTRACT

Epstein-Barr virus associated gastric cancer (EBVaGC) comprises approximately 10% of gastric carcinomas. Multiple factors contribute to tumorigenesis, including EBV driven hypermethylation of tumor suppressor genes, inflammatory changes in gastric mucosa, host immune evasion by EBV and changes in cell cycle pathways. The unique molecular characteristics of EBVaGC, such as programmed death ligand 1 (PD-L1) overexpression, highlight the potential for using EBV as a biomarker for response to immunotherapy. Few studies have reported benefit from immunotherapy in EBV positive cancers, and clinical trials investigating the impact of checkpoint inhibitors in EBVaGC are currently underway. This review provides the most recent updates on molecular pathophysiology, epidemiology, clinical features and treatment advances pertaining to EBVaGC.

Introduction

Until the late 1930s, gastric cancer was the leading cause of cancer death in the United States [1]. Today, it is the third most common cause of cancer-related mortality and the fifth most common cancer worldwide [2]. In the United States, it is the 14th most common cause of cancer, with approximately 10,960 deaths per year [1]. Gastric cancer has a significant socioeconomic, ethnic and geographic disparity, with highest rates in Eastern Asia, followed by Central and Eastern Europe, and lowest in North America and Western Africa [3]. Although the worldwide incidence of gastric cancer has declined over the last few years, the incidence of proximal gastric cancer has increased globally [4].

The overall 5-year survival rate in most parts of the world is dismal at 20% with median survival less than 12 months [5]. Gastric cancer's aggressive nature and its heterogeneity warrant the identification of new sensitive and specific biomarkers. To facilitate biomarker discovery and personalized treatment development, global efforts have been undertaken to molecularly classify gastric cancer. In 2014, The Cancer Genome Atlas (TCGA) network used six genomic and molecular platforms to comprehensively characterize 295 tumors into four molecular subtypes: Epstein-Barr virus (EBV)-positive tumors, microsatellite instable (MSI) tumors, genomically stable (GS) tumors, and tumors with chromosomal instability (CIN) [6]. In 2015, the Asian Cancer Research Group (ACRG) conducted gene expression data on 300 gastric cancers, leading to four different subtypes with prognostic data: MSI, microsatellite stable/epithelial-to-mesenchymal transition (MSS/EMT), MSS/TP53$^+$ and MSS/Tp53- [7]. The ACRG found that EBV infection occurred in 6.5% of overall patients, and more frequently in the MSS/TP53$^+$ subgroup, which had the second-best overall survival [7].

EBV-positive tumors comprised 9% of TCGA gastric cancer samples [6] and 6.5% of the ACRG samples [7]. EBV-positive tumors also exhibited a higher prevalence of DNA hypermethylation and elevated levels of programmed death ligands 1 and 2 (PD-L1/2) in TCGA samples. Although the ACRG analysis did not identify hypermutation among EBV-positive gastric cancers, it did find EBV to be more frequent in the MSS/TP53$^+$ subtype, with significant enrichment of PIK3CA and ARID1A mutations, and increased immune infiltrates [7].

These classification results suggest that EBV associated gastric cancers (EBVaGC) have a distinct tumorigenic profile, and present the opportunity for using EBV as a novel biomarker in gastric cancer for targeted treatment development. Limited progress has been made by adding targeted therapy to gastric cancer treatments. The addition of trastuzumab for gastric cancers with overexpression of human epidermal growth factor receptor 2 (Her2) (3+/2+ on immunohistochemistry or FISH positive) has had a modest improvement

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Table 1
Important host genes and their role in EBVaGC tumorigenesis.

Gene	Mechanism of tumorigenesis	Reference
Upregulated Genes		
ZEB1	Inhibits latent to lytic switch of EBV, enabling a longer latency duration	[26]
PIK3CA	Increases cell proliferation and survival by activating downstream PI3K/Akt pathway	[63]
PeBOW	A protein complex that enhances cell survival and ribosome biogenesis	[36]
PD-1/2	Suppresses immune surveillance and facilitate tumor development	[63]
JAK2	Stimulates cell proliferation, survival and differentiation	[63]
Bcl-2	Anti-apoptotic protein	[48]
Cyclin D1	Allows cell cycle progression through G1 phase	[48]
IHH	Increases metastatic potential through angiogenesis, Snail protein expression, as well as a decrease in e-cadherin and tight junctions	[23]
Downregulated Genes		
SSTR1	Expression is decreased by eightfold in EBVaGC, allowing cell proliferation, loss of apoptosis and invasion	[26]
PTEN	Loss of this tumor suppressor leads to PI3K/Akt pathway activation, and increased cell growth, angiogenesis, migration, loss of cell adhesion, and cell cycle regulation	[63]
ARID1A	Loss leads to enhanced tumor migration and lymphovascular invasion through downregulation of e-cadherin	[63]
P16	Loss leads to uncontrolled cell growth, and may induce expression of thymidine phosphorylase, which facilitates tumor angiogenic activity	[23]

in survival, with a median survival increase of 2.5 months based on the ToGA trial [8]. Recent meta-analyses have shown that gastric cancers with EBV positivity and microsatellite instability are most likely to overexpress PD-L1 [9]. Microsatellite instability (MSI-high) already serves as a biomarker in predicting utility of immune checkpoint inhibitors [10], where the PD-1 antibody, pembrolizumab, is FDA approved for use in MSI-high gastric cancers that have progressed on standard treatment. Most recently, results from the global phase II KEYNOTE-059 trial [11] showed an improved overall response rate to pembrolizumab in gastric cancer patients with overexpression of PD-L1. This led to the FDA approval of pembrolizumab for gastric cancer patients with overexpression of PD-L1 who have failed two or more lines of systemic chemotherapy. Nivolumab is another PD-1 antibody, which for the first time, has shown an overall survival benefit in gastric cancer patients in the ATTRACTION-2 phase 3 trial conducted in Japan, South Korea and Taiwan [12].

As the role of immunotherapy in gastric cancer gains momentum, the need for identifying biomarkers of response becomes crucial. Patients with EBVaGC could be the next subgroup most likely to benefit from immunotherapy. This review provides an overview of EBVaGC, the current clinical trials including EBVaGC and its implications for advancing personalized medicine in the care of gastric cancer patients.

EBV and cancer

EBV, also known as human herpesvirus 4 (HHV4), is a double-stranded DNA virus infecting over 90% of the adult population [13]. It was first discovered in 1964 by Tony Epstein and Yvonne Barr, when they used electron microscopy to identify herpesvirus-type particles in a subpopulation of Burkitt's lymphoma (BL) cell lines from African patients [13]. Since then, EBV has been recognized as the first virus to be directly associated with human cancer. It is currently categorized as a group-1 carcinogen due to its association with the development of a wide spectrum of cancers, including BL, post-transplant lymphoproliferative disorder (PTLD), Hodgkin and non-Hodgkin lymphomas, nasopharyngeal carcinoma (NPC) and more recently, gastric carcinoma [14].

Defined by the presence of EBV in gastric cancer cells, EBVaGC has an annual incidence of 75,000–90,000 cases per year, representing the largest subpopulation among EBV-related tumors [15]. EBVaGC was first reported by Burke et al. in 1990 using polymerase chain reaction (PCR) in gastric carcinoma cells resembling lymphoepithelioma [15]. In 1992, in situ hybridization technique allowed Shibata et al. to identify EBV in 16% of gastric adenocarcinoma samples by localizing EBV-encoded small RNA 1 (EBER1) [16]. Its etiological involvement in gastric tumorigenesis is strongly suggested by the detection of monoclonal episomes and uniform presence of EBER in tumor cells but not in the adjacent normal mucosa [16].

Pathophysiology and tumorigenesis

EBV is considered a direct transforming pathogen [17] by expressing its own regulatory genes affecting host cell cycle pathways [18]. It enters epithelial cells from the oropharynx and subsequently spreads to the lymphoid tissues where it infects B lymphocytes [19].

After primary infection via the oral route, EBV establishes a lifelong virus carrier state, called latent infection, where it persists as an episome within the nucleus [20]. During its latency cycle, it constitutively expresses a limited set of latent gene products known as: EBV nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C and EBNA-LP) [21], EBV-encoded small RNAs (EBERs) 1 and 2, latent membrane protein (LMP 1, 2A and 2B) and 40 microRNAs from BamHI-A rightward transcripts, known as BARTs [22]. Depending on the various combinations of these gene products, four latency patterns have been classified: latency Ia, Ib, II, and III [23]. EBVaGC belongs to latency I, which is limited to EBERs, BARTs and EBNA1 [23]. The absence or presence of LMP2A distinguishes latency type Ia or Ib, respectively. In over 50% of EBVaGC cases, LMP2A is expressed [21].

EBV infection induces extensive CpG island methylation [23] within approximately 18 weeks of infection [24], and is significantly correlated with CpG island methylator phenotype (CIMP)-high status [25]. This characterizes an important pathogenic mechanism, known as EBV-specific methylation epigenotype [24]. It is currently under investigation whether the host cell initiates genome-wide methylation as a defense mechanism, or whether EBV directly begins the process. LMP2A is reported to induce host DNA methyltransferase 1 (DNMT1) [24] or DNMT3b [26] overexpression and initiate genome-wide methylation. Overall, 886 genes are known to be methylated [26], with downregulation of approximately 216 genes [23]. Table 1 lists important host genes that are affected by EBV infection, and their role in tumorigenesis.

A comprehensive analysis of promoter methylation status of 51 gastric carcinoma cases was conducted by Shinozaki et al. [27], who subsequently classified gastric carcinoma into three epigenotypes characterized by different sets of methylation genes: EBV + /extensively high-methylation, EBV − /high-methylation and EBV − /low-methylation. Methylated genes specific for the EBV + subtype included CXXC4, TIMP2 and PLXND1. COL9A2, EYA1 and ZNF365 were highly methylated in EBV + and EBV − /high-methylation subtypes, whereas AMPH, SORC33 and AJAP1 were frequently methylated in all epigenotypes. They discovered that EBVaGC had approximately 270 genes which were uniquely methylated. Interestingly, MLH1 was frequently methylated (46%) in the EBV-/high-methylation type, whereas none of the EBVaGC cases showed MLH1 methylation. Different methylation