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Review

Epstein-Barr Virus and DNA Methylation in Gastric Cancer

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Abstract

Gastric cancer (GC) is the fourth most common type of malignity and the second leading cause of cancer death worldwide. Infectious agents such as Helicobacter pylori (H.pylori) and Epstein-Barr virus (EBV) play important role in the etiology in GC. EBV is a double-stranded DNA virus, approximality 184 kb in size, included in Herpes virus family and is the first virus determined in human neoplastic cell. EBV associated gastric cancer (EBVaGC) constitutes approximately 10% of whole GC in wordlwide. DNA hypermethylation in promotor region of tumor supressor genes is seen frequently in the genetic basis of EBCaGC. It is not a clear subject how EBV infection induce the methylation. In this review, the current literature about the gene promotor region hypermethylation in EBVaGCs will be revised.

Keywords: Gastric cancer, Epstein-Barr virus, DNA hypermethylation

Introduction

Gastric cancer (GC) is a malignancy that is in fourth place in the world in terms of incidence and in second place in cancer-related deaths (1, 2). GC, which is a malignancy having a multifactorial etiology, shows a heterogeneous behavior in biological and genetic aspects (3). The main reason for the pathogenesis is chronic Helicobacter pylori (H. pylori) infection. The International Cancer Research Agency accepted H. pylori as a class 1 carcinogen in 1994. This classification was updated and approved again in 2009 (3). The other infectious agent associated with gastric carcinogenesis is Epstein–Barr virus (EBV).

Though early diagnosis in recent years, improved surgical techniques, and improvements in perioperative care conditions have influenced the clinical course of GC in a positive way, GC is still a major problem because of its high prevalence, poor prognosis, and limited treatment options (2). Due to the advanced stage of disease at diagnosis, 5-year survival is only 20%–30% (4). The fatality rate of GC is higher than colon, breast, and prostate cancers that are common.

The prognosis of gastric cancer depends on its stage at diagnosis and the selection of appropriate treatment strategies. The identification of new molecular markers in the early diagnosis of cancers and the therapeutic strategy will positively affect the clinical course of the disease. This will be possible only with the understanding of the biological behavior of the tumor. In this compilation, recent literature will be reviewed about the relationship between DNA hypermethylation observed in EBV and in GC.

1. DNA methylation

Cancer occurs by the accumulation of genetic and epigenetic changes over a long period. DNA methylation is common in cancer and is divided into two categories: “whole genome hypomethylation” and “regional hypermethylation” (5).

Genome hypomethylation is evaluated as the decrease in 5-methylcytosine content in the entire genome (5). This is particularly seen in repeat sequences, which constitute more than 40% of the genome and are quite methyl under normal conditions. Genome hypomethylation seen in almost all cancers increases the progression of cancer by causing genomic instability (5).

DNA methylation occurs by the addition of a methyl (CH_3) group to the fifth carbon of cytosine residues in CpG islands (2, 6). CpG islands are located in the promoter regions of about half of all of the genes in the genome and are regions rich with guanine and cytosine. Physiologically, DNA methylation is seen in events such as X chromosome inactivation, differentiation in the stage of embryogenesis, and genomic imprinting (6).

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DNA methylation is catalyzed by the enzymes called DNA methyltransferase (DNMT) (6). While the continuity of DNA methylation occurs with the enzyme DNMT1, DNMT3A and DNMT3B enzymes serve in de novo DNA methylation (7). Transcription is suppressed by the hypermethylation of the gene promoter region. This is one of the major reasons leading to function loss in tumor suppressor genes (5). Promoter-region hypermethylation is commonly seen in the early stages of cancer (4). Methylation characteristics of the tumor DNA can be used as biomarkers of screening, diagnosis, prognosis, and treatment in cancer. In gastric carcinogenesis, promoter-region hypermethylation in certain cancer-associated genes is one of the frequently seen genetic changes.

2. EBV

Epstein–Barr virus is a double-branched DNA virus that is about 184 kbp in size and is part of the gamma-herpes virus family (7). EBV was first found in 1964 in vitro in the cells derived from human Burkitt lymphoma cell lines. EBV genome was detected in 1990 using polymerase chain reaction (PCR) for the small EBV-encoded RNA 1 (EBER1) and in situ hybridization (ISH) (8).

Epstein–Barr virus infects 95% of the world population. While the vast majority of EBV infections stay asymptomatic, hematopoietic, mesenchymal, and epithelial tumors develop in a small proportion of infected people. Tumorigenesis occurs by means of EBV viral proteins and microRNAs (miRNAs) (9). EBV-associated malignancies can be classified into three categories (10). This classification and some examples of malignancy are as follows:

- Lymphoproliferative disorders:
  Diffuse large B-cell lymphoma, post-transplantation lymphoproliferative disorders, plasmablastic lymphoma, Hodgkin's lymphoma, Burkitt lymphoma, lymphoma associated with HIV infection.
- Epithelial cancers:
  Nasopharyngeal carcinoma, lymphoepithelial-like carcinoma, gastric carcinoma.
- Sarcomas and other soft tissue tumors:
  Inflammatory pseudotumor variant of follicular dendritic cell sarcoma, post-transplantation smooth-muscle tumors

Epstein-Barr virus primarily enters through the oropharyngeal epithelial cells with saliva (10). It infects people with a normal healthy immune system and continues to live as latent in memory B cells throughout life. In order to escape from the host immune surveillance, it limits itself by expressing 1-2 viral proteins in these cells. This is defined as the latent 0/1 stage.

It was shown to express at least three virals, including the latent membrane protein 1 (LMP-1) in nasopharyngeal carcinoma (NPC) and some EBV-associated malignancies such as Hodgkin lymphoma. This is called the type 2 latent phase. It is not exactly clear how these malignancies with viral infection can escape from the immune system. The in vitro infection of B lymphocytes results in immortalization and the continuation of lymphoblastoid cell lines. In these cells, many different EBV latent products are expressed, including at least six EBV nuclear antigens (EBNAs), three latent membrane proteins (LMPs), and two small RNAs (EBERs) not encoding protein. Several microRNAs were also shown to be expressed. This is called type 3 latent or growth program (11).

The theory that is gaining considerable acceptance in oncogenesis is that neoplastic cells remain in the stage of cellular growth or maturation when the transformation occurs (10).

3. EBV and Gastric Cancer

The oncogenic role of EBV in different malignancies varies according to the general immune system of the host and cell type (12). Almost all of the NPC are associated with EBV and are particularly common in South China and South Asia.

Regional and racial differences are not seen in EBV-associated GCs (EBVaGC). EBVaGCs show a uniform distribution worldwide, without any regional accumulation. EBVaGCs constitute 10% of all gastric cancer cases, but it is not an endemic disease (13). EBV infection is detected in two types of GC as 16% of gastric adenocarcinomas and 89% of lymphoepithelioma-like gastric carcinomas (13). EBVaGCs show the characteristics of monoclonal proliferation of tumor cells bearing EBV (8).

Epstein-Barr-associated GCs have some different clinicopathological features compared to EBV-negative GCs. The characteristics of EBVaGCs can be sorted as follows (9).

- Age → young
- Gender → mostly male
- Prevalence → 10% of the GCs
- Accompanying findings → smoking
- Settlement → proximal part of the stomach
- Clinic → thickening of the gastric wall, ulcers, little involvement of the lymph node
- Histology → atrophic gastric, lace-like appearance of the mucosa, adenocarcinoma ranging from moderate to bad
- Prognosis → long lifetime

Epstein-Barr virus infects host gastric epithelial cells either directly or indirectly. The viral envelope interacts with glycoprotein BMRF-2 cellular β1 integrins in direct infections. Subsequently, the viral protein gH/gL binds to cellular αvβ6/8 integrins and aims to facilitate the fusion of epithelial cell membranes with a viral envelope (9).

Epstein–Barr virus preferentially infects B lymphocytes. In B cell invasion, EBV envelope glycoproteins gp 350/220 bind to B-cell receptor CD21 and/or CD35. Viral glycoprotein gp24 simultaneously interacts with human leukocyte antigen (HLA) class II molecules on the B cell membrane that enables EBV to enter B cells (9). The mechanism of epithelial cell invasion has not been fully explained.

EBER, EBNA 1, BARTs, LMP2, and BARF1 genes are expressed in EBVaGCs. LMP1 and LMP2 are oncogenic EBV proteins that play an important role in tumor transformation of the epithelial and lymphoid cells. LMP1 is not often expressed in EBVaGCs or is expressed at low levels (14). Half of EBVaGCs express LMP2. LMP2 plays an important role in the oncogenic process of EBVaGCs. LMP2A activates a variety of cellular signals, including the JAK/STAT3 and PI3K/AKT signaling pathways. LMP2A regulates transcriptional DNMT1, DNMT3B, and BMI1 expression in the protein level (14).

The EBER1-ISH method is the most accurate method to identify EBV infection. EBER1-ISH application to the gastric mucosal biopsies of patients who have upper gastrointestinal endoscopy is very useful for the diagnosis of EBVaGC. The value of serum antibodies

is high against EBV early antigen and EBV capsid antigen in patients with EBVaGC. However, the EBNA1 antibody titer does not show a significant difference between healthy and sick people (8).

4. Genetics and DNA hypermethylation in EBVaGC

Gastric cancer exhibits quite a complex and heterogeneous structure genetically. Chromosomal alterations and microsatellite instability, which are rather common in solid tumors, are not among the frequently seen genetic changes in EBVaGCs (1). Chromosomal loss or gain was shown to be very low in the analyses made with the method of comparative genomic hybridization (CGH).

In whole genom studies comparing the promoter methylation between the GC cell lines infected and non-infected by Epstein-Barr virus, it was observed that, for the examined hundreds of genes, such as cell adhesion molecules, the wnt signaling pathway and mitogen-activated protein kinases were hypermethylated after EBV infection (9).

Geddert et al. (15) reported in their study that p16, the p14, and APC genes in EBVaGCs show more methylation characteristics than EBV negative GCs. In EBVaGC, the methylation frequency was stated to be more in the TP73 gene by Ushiku et al. (16), in the somatostatin receptor 1 gene by Zhao et al. (17), and in the TP73, BLU, FSD1, BCL7A, MARK1, SCRN1, and NKX3.1 genes by Okada et al. (18).

In the study of Zhao et al. (19), when EBV positive and EBV negative GC cell lines were compared (confirmed with the EBER ISH), DNMT3B mRNA and protein expression were demonstrated to be higher in EBV positive cell lines in comparison to EBV negative cell lines.

Epstein-Barr virus latent genes disrupt the regulation in signaling pathways, and gene expression then initiates tumor formation, DNA methylation may be affected. LMP1 is a transmembrane protein expressed in the latent I/II phase and activates various pathways (7). Tsai et al. (20) reported that LMP1 disrupts the regulation of the CDH1 gene by upregulating DNMT1, DNMT3A, and DNMT3B in the NPC cell line. Hino et al. (21) reported that LMP2 increases DNMT1 expression 5-fold in GC through STAT3 phosphorylation, and PTEN gene methylation increased 5-fold in 96 h.

These results suggest that EBV latent genes could activate the DNMT enzymes and can cause methylation in the gene promoter region.

Although LMP2A is expressed in EBVaGCs, it is an interesting subject that LMP2A antibodies are usually found negative in EBVaGC patients (8).

5. The clinical application of EBV-associated methylation

Epstein-Barr virus-associated methylation is clinically important. Because EBV-positive tumors have different methylation characteristics, it should be regarded as a sensitive measurement method in early diagnosis. Because DNA methylation occurs in the early stages of carcinogenesis, precancerous lesions are important. In the early diagnosis of GC, methylation characteristics specific to cancer may be examined in the gastric washing liquid. Serum samples may also be used for early diagnosis (2).

The disrupted DNA methylation in cancer is a reversible event. Today, enzymes that catalyze epigenetic modifications have become important pharmacological targets. In particular, the demethylating agents that inhibit the DNA methyltransferase enzymes and reverse the tumor suppressor gene silencing generate a potential strategy for antineoplastic treatment in GC (9).

Conclusion

Epstein-Barr-associated GC which is a malignancy seen worldwide, exhibits different clinicopathological features from those with EBV negative, and this suggests different molecular mechanisms involved in carcinogenesis. The biological basis of DNA hypermethylation seen in EBVaGC remains unclear. Further studies should continue to enlighten the subject. As in other malignancies, understanding of the molecular structure in GC will also bring forward new tumor biomarkers for early diagnosis and treatment.

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