Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 327-336 Online ISSN: 2537-0979

REVIEW ARTICLE

Correspondence between Epstein Barr Virus Infection and Programmed Death Ligand 1 in Bladder Cancer Patients

[1]Amira E. Elzalouey*, [1]Sanaa Mohei El-dien, [2]Sahar T. Mohamed, [1]Doaa T. Masallat, [3]Ahmed M. Othman

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University and Horus University, Mansoura, Egypt
[3]Urology and Nephrology Center, Mansoura University, Mansoura, Egypt

Key words:
Epstein Barr Virus, Bladder cancer, Programmed death ligand 1

Corresponding Author:
Amira H. Elzalouey
Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Tel.: 01000970403
amiraelzalouey@gmail.com

ABSTRACT

Worldwide, bladder cancer (BC) remains a substantial health challenge, with immune system interactions having an important role in its pathogenesis and progression. Epstein-Barr virus (EBV) has been related to several malignancies, yet its role in BC is not well-understood. Programmed cell death-ligand 1 (PD-L1), an immune checkpoint molecule, has the ability to contribute to tumour immune evasion and has become a major target for immunotherapy. This review explores the potential association between EBV infection, bladder cancer, and the expression of PD-L1. We summarize current evidence on EBV's oncogenic mechanisms, its potential involvement in bladder carcinogenesis, and its influence on PD-L1 regulation. Additionally, we discuss the implications of EBV-associated immune modulation in BC and its potential impact on immunotherapeutic strategies, particularly checkpoint inhibitors. Understanding the interplay between EBV, BC, and PD-L1 may provide new insights into tumour immune escape mechanisms and novel therapies, ultimately improving patient outcomes.

INTRODUCTION

Epstein Barr Virus is a double-stranded DNA virus. It belongs to the gamma herpes virus subfamily that exclusively infects more than 90% of general population globally. It is predominantly spread through saliva and transmitted to B cells through the oropharyngeal epithelium where a long-term latent infection becomes established. This suggests that EBV can escape the immune surveillance mechanism. Generally, escaping the immune surveillance is the hallmark of malignancy[1].

In most EBV-associated cancers, EBV is latent and expresses a small set of proteins called Epstein Barr virus transcription programs include viral nuclear antigens (EBNAs), latent membrane proteins (LMP), and un-translated RNA called EBV encoded RNA (EBER)[2] .

Globally, BC is the 7th most common malignancy. In Egypt, it is the 3rd most common malignant tumour after breast cancer and hepatoma. BC is also more common among males compared to females. Several risk factors have been identified to have roles in BC development. They include smoking, exposure to chemicals e.g. aromatic amines, and bilharziasis. Furthermore, certain viral infections (e.g. Human papilloma virus and EBV) have been associated with tumorigenesis[3].

EBV has been found to be associated with many cancers in humans, such as classical Hodgkin's disease and African Burkitt's lymphoma. In such cancers, EBV genome and related proteins were identified in all cancer cells indicating their pathogenic roles[4].

Moreover, EBV has been reported to be linked to other malignancies such as breast, bronchogenic carcinoma and hepatoma. In Taiwan, a study detected EBV-encoded RNA in BC cells[5]. However, it is still not clear if EBV contributes to BC development. It is still needed to confirm the presence of EBV in BC patients in different countries and to look into its possible relevance[1].

Programmed death 1 (PD-1) is expressed on activated T lymphocytes, natural killer (NK) cells, B cells, and dendritic cells (DCs). More specifically, programmed death ligand 1 is expressed by tumour cells or antigen presenting cells (APCs). The PD-1/PD-L1 complex inhibits the proliferation and activity of killer T- lymphocytes[6]. Many reports showed that membrane-bound PD-1 and PD- L1 have also soluble forms. Serum levels of soluble PD- 1/PD-L1 have been found to help predict clinico-pathological characteristics, response to therapy, and survival among cases with cancers[7].

Gaining more knowledge about the interactions between tumors and the immune system has helped the advent of "immune-checkpoints inhibitors" as a therapeutic strategy for BC[8].Targeting the PD1/PD-L1 axis with immunotherapy is a promising field in anti-

cancer therapy. Though inhibiting the immune checkpoint proteins, the tumour cell can no longer resist the immune response together with restoration of an effective T-cell response against tumour cells. It was shown that EBV infection upregulates PD-L1 via complex mechanisms[9].

Epstein-Barr Virus

Epidemiology

Epstein-Barr virus is a highly prevalent gamma herpesvirus. In USA, the prevalence of EBV in children and adolescents aged the 6 to 19 years is 66.5%. In children 6-8 years old, the prevalence of about 54%, compared to 82.9% in adolescents 18-19 years old. EBV was the 1st human oncogenic virus to be discovered and has been associated with many malignant tumours. Worldwide, EBV has been reported to be responsible for about 1.5% of all malignancies in humans[10].

Taxonomy, and structure of EBV

Taxonomy

EBV has recently been classified as a member of the Orthoherpesviridae family, subfamily Gammaherpesvirinae. There are 8 herpesviruses that infect the human and they are classified into α, β, and γ subfamilies. Alphaherpesvirinae include herpes simplex virus types 1 and 2, and varicella-zoster virus. Beta-herpesvirinae include cytomegalovirus and roseolovirus. The gammaherpesvirinae include EBV and Kaposi's sarcoma-associated virus[11].

Structure of virion

EBV is a DNA virus. It has a toroid-shaped protein core wrapped with DNA, a nucleocapsid with 162 capsomers, a protein tegument between nucleocapsid and the outer virus envelope. The latter has external EBV spike glycoproteins [12].

Pathogenesis of Epstein-Barr Virus infection

Primary infection and lytic phases

The tropism of EBV virion is determined by the glycoproteins of the envelope and thus may vary depending upon the host cell. Oropharyngeal epithelial cells and B lymphocytes are the major cells infected with EBV. Epstein-Barr virus is predominantly transmitted via saliva, with the oropharyngeal B-lymphocytes and epithelial cells are initially infected with the virus[13].

Entry to B cells

For viral attachment, EBV glycoprotein gp350/220 binds the complement receptor 2 (CR2/CD21) on B-lymphocytes. Also, the complement receptor 1 (CR1/CD35) serves as an additional attachment factor and facilitates entry of EBV into CD-21 negative cells as immature B cells. After attachment, glycoprotein gp42 interacts with HLA class II on B-lymphocytes. This brings EBV closer to host cells and triggers the core fusion machinery composed proteins (gH, gL and gB) to interact with endosomes[14].

Entry to epithelial cells

Epithelial cells have no CD21 and HLA-II and as a result, when EBV infects these cells, the EBV BMRF2 binds to intergrins and then the gH/gL binds to integrins and ephrin receptor A2. This activates gB and fusion of virus envelope to plasma membrane[15].

After fusion, the tegument with nucleocapsid becomes released into cell cytoplasm. After dissolution of tegument proteins, EBV injects its genome into the nucleus to initiate viral replication via DNA polymerase. The viral genome encodes more than 80 gene products. The latter facilitates the replication of EBV and production of structural components during the lytic phase[16].

The sequence of viral gene expression involves 3 steps: immediate early (IE), early (E) and late (L). IE lytic genes, including *BZLF1* and *BRLF1*, encode for lytic cycle transactivators. E lytic genes, e.g. *BNLF2a*, are largely linked to viral replication and metabolism. L lytic genes, e.g. *BCRF1*, might have an association with immune evasion[17].

Infected B-lymphocytes become attached by cytotoxic T-cells, which release viral products into host circulation and control the number of infected B-lymphocytes. Similarly, infected memory B-lymphocytes are released into host blood stream where they remain in the latent or lytic phase[18].

Latent infection phase

Epstein Barr virus can remain in a latent phase. Following infection, the linear DNA of the virus changes into circular DNA in the nucleus "circular episomes". These episomes attach to host chromatin via EBNA-1, replicates and are transmitted to daughter cells[19].

During this phase, the virus expresses only a small group of genes and non-coding RNAs. These include 6 EBNAs (EBNA-1, EBNA-2, EBNA-3 A, EBNA-3B, EBNA-3 C, and LP); 3 LMPs (LMP-1 and LMP-2 A/B); and 2 types of non-coding RNA that are not translated into proteins, EBERs and miRNAs[20]. EBV can establish many latent gene expression types (Types 0, I, II and III); this depends upon the type of infected cells and whether they are resting or proliferating[21].

The latent EBV genome is propagated in memory lymphocytes during the Latency I, induces differentiation of B lymphocytes throughout the Latency II, induces the transformation of naive B lymphocytes throughout the Latency III, while all expressions are stopped in the memory B- lymphocyte pool during the Latency 0[22].

Lytic reactivation phase

Infected memory B-lymphocytes during the latent phase are not reactivated and rarely differentiate into plasma cells, which can trigger virus reactivation following cell lysis, thus the virus enters the lytic phase. *BZLF1* and *BRLF1*, IE lytic genes, encode transcription factors ZTA/BZLF and RTA/BRLF1, which have a key role in virus reactivation[23].

During lytic phase, BZLF1 and/or BRLF1 binds the origin site of virus DNA, triggers their own and each other's promoters (Zp and Rp), and eventually activates E lytic gene. When viral replication is completed, the new genome becomes linearized and then is packaged into previously capsid particles to produce nucleocapsids. The latter is released from cell nucleus to enter the cytoplasm where viral proteins coat them forming teguments. Finally, the virus acquires its envelope. The cellular vesicles are fused (via exocytosis) with plasma membrane and release infectious virions[24].

EBV transmission & associated diseases

Transmission

Epstein-Barr virus transmission occurs mainly through saliva. However, deep kissing, food sharing, breast feeding, body fluids, and transplantation of EBV-infected organs can cause viral transmission from one host to another[25].

Primary EBV-related disorders

Infectious Mononucleosis (IM) is a common manifestation of EBV infection. It develops in about 35-50% of those having their first EBV infection during adolescence. The classic clinical features are fever, lymphadenopathy, and herpangina. Other symptoms such as headache, fatigue, rash, jaundice, hepatomegaly and splenomegaly have been also reported[26].

Persistent EBV-related disorders

Chronic active EBV is a progressive illness that can a last for more than 90 days. Following EBV infection, there may chronic or recurrent IM-like symptoms along with failure of body organs, including hepatic failure, multiple lymphadenopathies, hepatomegaly, splenomegaly, retinal inflammation, pneumonia, and vaccinia-like rash[27].

EBV Carcinogenesis

The WHO has classified EBV as group 1 carcinogen. EBV infection is associated with more than 200,000 new malignancy cases annually and cause 150,000 mortalities globally[28].

A) Expression of viral proteins during EBV latent phase have an important role in tumorigenesis: latent proteins are necessary for B-cell transformation, and these proteins have roles not only in inducing oncogenes' overexpression, tumour suppressors' silencing and cell cycle migration, but also in regulating adhesion[29].

Latent membrane protein 1 & 2A (LMP1 & 2A) is highly oncogenic protein that triggers multiple pathways such as LMP1-mediated p53 degradation. In addition, it alters the expression of miRNA, promoting tumorigenesis. LMP2A, mimic B cell receptor, impairs apoptosis and works in a synergism with oncogenes to increase survival and proliferation. Additionally, EBV nuclear antigen 1 (EBNA1) is another latent protein implicated in tumor formation. EBNA1 upregulates the survivin (an anti-apoptotic protein) and interacts with ubiquitin-specific protease 7 (USP7) to reduce P53 levels. Moreover, EBV nuclear antigen 3A/C (EBNA 3A/C) interact to induce tumorogenesis via several mechanisms, such as inhibiting BCL2/apoptosis and cyclin dependent kinase[30].

B) EBV microRNAs: EBV genome encodes 44 miRNAs. The latter has been found to have a key role in EBV-related tumorogenesis, including cellular proliferation, apoptosis, invasion, and transformation. Furthermore, miRNAs can target immune-related genes, enabling the infected cell to escape immune system[31].

Diagnostic methods for EBV detection

The detection of EBV within a certain tumor suggests that its role in the clonal expansion in EBV-related disorders. Thus, EBV can serve as a biomarker for diagnosis and assessment of tumour spread and also for treatment monitoring[32].

Classical techniques

Electron Microscopy (EM)

EM provides a high-resolution image of viral specimens and virion's structure. EBNA1 is essential for maintaining chromosomal expression during the latent phase and expression in EBV-associated cancers. EBNA1 can serve as a biomarker for EBV detection using EM[33].

Viral Culture

Viral culture is a sensitive method that commonly utilized to diagnose Herpesviridae. It is considered the gold standard when compared to other diagnostic methods. Accurate viral culture is important because of its slowness, lower sensitivity, increased contamination rate, as well as the possibility of virus release within the lab. Therefore, serology and molecular assays are favored[33].

EBV-encoded small RNAs – In Situ Hybridization (EBER-ISH)

EBER-ISH is the best method for detection of latent EBV in tissues. EBER transcripts have been found to be expressed in both EBV-infected cancer cells and lymphoid tissues of IM individuals[34].

EBV Serology

Three serological methods are used for detection of EBV: 1. Indirect Fluorescent Antibody (IFA) Assay "gold standard"; 2. Enzyme immunoassay assays, such as enzyme-linked immunosorbent assay and anti-EBV antibody luminescence, and 3. Western blot analysis[37]. Epstein Barr Virus antibodies target the antigens of lytic and latent phases in humoral response. The major EBV-antibodies are EBV anti-viral capsid antibody (Anti-VCA) IgM, EBV anti-viral capsid antibody (Anti-VCA) IgG, and Anti-EBV (IgG) early antigen[35].

Molecular methods

For those at risk of EBV-related lymphoproliferative diseases and EBV-associated cancers, EBV-DNA detection using molecular assays is of value in diagnosis and management. Quantitative estimation of EBV-DNA can distinguish healthy EBV carriers from those having EBV-related disorders[36].

Treatment

There is no specific treatment for EBV-related disorders and adverse events. The main therapy is symptomatic treatment. The antiviral drug acyclovir inhibits EBV DNA polymerase but not the host-cell polymerase. Thus, though acyclovir interferes with lytic replication in host cells, it exerts no effects on EBV when lymphocytes divide and utilize host-cell polymerase for EBV-DNA replication[37].

Bladder Cancer

Worldwide, BC is the 6th most common malignant tumor in both sexes and the 4th most common malignancy among males. The bladder's wall is made of multiple layers which include 1) the inner epithelium (urothelium); 2) subepithelial connective tissue (lamina propria), which contains vasculature, lymphatics, and a thin incomplete muscularis mucosa; 3) muscularis propria (detrusor muscle), which consists of inner and outer smooth muscles; and 4) adventitia (loose connective tissue)[38].

Generally, 90% of BC cases arise from the urothelium. Although localized urothelial cancers have a good prognosis, invasion of smooth muscles has been linked to a significant drop in survival rates. Squamous cell carcinoma occurs in 5% of cases and is more prevalent in African countries. This type of BC is likely linked to bilharziasis[39].

Risk factors

Sex; worldwide, BC is 4-folds more common among males compared to females. This may be attributed to differences in smoking rates, exposure to certain chemicals in their work, hormonal factors, alcohol consumption and the effect of Y chromosome[40].

Age; BC is mainly a disease that affects older adults, with > 90% occurs in those aged >55 years, and an average age of 73 years at diagnosis. BC can rarely affect pediatric population and young adults, and it is often of low-grade and non-invasive[41].

Hereditary and Genetic Factors; although BC is not typically considered as hereditary, some syndromes predispose to it. For instance, Cowden's syndrome which id due to an inherited change in the PTEN gene (a tumor-suppressor gene) can predispose to many cancers including BC[42].

Smoking; accounts for about 50–65% of new BC cases annually. Tobacco smoke contains the carcinogenic polycyclic aromatic hydrocarbons which promote inflammatory response, and genetic mutations. These mutations activate oncogenes or inhibit tumor suppressor genes, resulting in cancer development[43].

Environmental and Occupational Exposures; exposure to certain chemical compounds at the work environment is considered as another risk factor for BC. Polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, and aromatic amines may enhance BC risk. Arsenic exposure also increases the risk of BC[44].

Pathogens; in northern Africa, infection with the parasite *Schistosoma haematobium* is a relatively unique risk factor of BC. The parasite infects the individual through his/her skin while swimming in a water containing cercariae and, after being matured in the host liver, it deposits eggs in urinary bladder. Eggs' calcification together with the resulting inflammation of the bladder can lead to squamous cell carcinoma[45].

Classification, grading, and Staging

In 1973, urothelial tumours were classified into 3 grades: G1, G2, and G3. In 2004 and later in 2016, this classification was updated and tumours were reclassified based on a grading system into low-grade tumours, encompassing G1 and part of lesions classified as G2; and high-grade tumours, including "more aggressive" G2 and characterized as G3 lesions. In addition, a new grade was introduced which is "papillary urothelial tumor of low malignant potential", characterizing abnormal growth lesion that does not form a tumour, with low malignant potential and previously classified as G1[46].

Clinical picture

About 75% of BC cases present with painless, macroscopic hematuria. The patient may also present with microscopic hematuria. The patient presents with lower urinary tract symptoms such as dysuria, frequency or urgency, predominantly in carcinoma in situ. More advanced tumours might cause obstruction of the upper urinary tract and pain[47].

Diagnosis

A patient with hematuria should be physically examined including rectal and vaginal examination to detect any pelvic mass suggestive for a locally advanced tumor[50]. Moreover, Urine cytology is a cost-effective method that helps diagnose high-grade BC. It has low sensitivity and high specificity[49].

Cystoscopy is the gold standard method for BC diagnosis. White-light cystoscopy can detect BC but some lesions may be missed e.g. Carcinoma-in-situ (CIS). Advanced technologies, including narrow-band imaging and photodynamic diagnosis have enhanced the detection of CIS. If the mass could be detected by cystoscopy, this is followed by tumor assessment under anesthesia at the time of transurethral urethral resection of bladder tumor (TURBT), however there is risk of under-staging or over-staging[50].

Table: TNM staging of BC, developed by the American Joint Committee on Cancer (last updated in 2023; 9th edition).

Primary tumour (T)	
TX	Primary tumor cannot be assessed
T0	No evidence for primary tumor
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumour"
T1	Lamina propria is invaded
T2	Muscularis propria is invaded
pT2a	Superficial muscularis propria (inner half) is invaded
pT2b	Deep muscularis propria (outer half) is invaded
T3	Perivesical tissue is invaded
pT3a	Microscopically
pT3b	Macroscopically (extravesical mass)
T4	Tumor invasion of prostatic stroma, seminal vesicle, uterus, vagina as well as pelvic and abdominal walls
T4a	Tumor invasion of prostatic stroma, uterus, vagina
T4b	Tumor invasion of pelvic and abdominal wall
Regional lymph nodes (N)	
Regional lymph nodes (LNs) Including primary and secondary drainage regions. Other LNs above the aortic bifurcation are considered distant LNs.	
NX	LNs cannot be assessed
N0	Absence of metastases to LNs
N1	Single regional metastasis to LNs in the true pelvis (perivesical, obturator, internal and external iliac, or sacral)
N2	Multiple regional metastases to LNs in the true pelvis (perivesical, obturator, internal and external iliac, or sacral)
N3	Metastases to common iliac LNs
Distant metastasis (M)	
MO	No distant metastasis
M1	Distant metastasis
M1a	Distant metastasis only to LNs beyond common iliac LNs
M1b	Non–lymph node distant metastasis

Treatment

The treatment of BC depends on risk stratification and extent of invasion of muscle layer. TURBT is usually used for low-grade NMIBC. Immediate intravesical chemotherapy (mitomycin C) within 24 hours of TURBT could decrease recurrence rates in some cases. For tumours of intermediate or high risk of progression, adjuvant intravesical therapy is recommended. Treatment with Bacillus Calmette–Guérin (BCG) after TURBT has more efficacy in preventing the recurrence of high-grade Ta and T1 cancers compared to TURBT alone or TURBT + chemotherapy. The BCG is instilled intravesically in an induction course composed of six weekly instillations followed by a maintenance course (three weekly instillations at 3, 6, 12, 18, 24, 30, and 36 months)[51].

Muscle invasive bladder cancer (MIBC) tumours are either indicated for radical cystectomy, with or without systemic chemotherapy with platin-based regimens, or radiation therapy in some patients. About half of MIBC patients might have micro-metastasis at the time of radical cystectomy and cisplatin-based chemotherapy is indicated in these patients[52].

In 2016, the FDA approved immunotherapy using checkpoints inhibitors (ICIs) directed to PD-1 or PD-L1 for metastatic cases that are unresponsive to platinum-based chemotherapy. ICIs are used only for cases with PD-L1 positivity, advanced BC, and to cases not eligible for platinum-based chemotherapy[53].

Programmed Death protein 1 / Programmed death Ligand 1

Immune checkpoint molecules

Immune checkpoints is defined as ligand-receptor pairs that inhibit or stimulate the immune response. They maintain self-tolerance, and modulate the length and magnitude of immunologic response. Though the primary function of cancer cells-associated immune

checkpoint molecules is mediating immune evasion, they play significant roles in maintaining the behaviors of the cancer, such as self-renewal, metastasis, epithelial-mesenchymal transition, resistance to drugs, anti-apoptosis, angiogenesis, or increased energy metabolism[54].

There are 2 groups of immune checkpoint molecules; co-stimulatory checkpoints (CD27, CD28, CD137, ICOS, 4-1BB and OX-40) which activate T lymphocytes, and co-inhibitory molecules (PD-1, CTLA-4) which inhibit the activation of T lymphocytes to avoid exaggerated inflammatory response and autoimmunity. However, in the cancer microenvironment, some inhibitory molecules undergo overexpression contributing to tumor-promoting immunosuppression, thus the inhibition of negative checkpoints can be valuable for boosting the immune response against the cancer cell[55].

PD-1 expression

PD1 "also known as PDCD1 and CD279" is a type I transmembrane glycoprotein. Many cells express the PD1 such a NK cells, monocytes, DCs, NKT cells, T-lymphocytes, and B-lymphocytes. It is expressed on double-negative αβ and γδ T-lymphocytes in thymus and induced on peripheral T-lymphocytes and B-lymphocytes after their activation[56].

Expression of PDL1 and PD-L2

Programmed Death Ligand 1 and 2 (PDL1 and PD-L2) are type I transmembrane glycoproteins. PD-L1 is expressed by all haematopoietic cells, several types of non-hematopoietic cells (e.g. endothelial, epithelial, and mesenchymal stem cells), and some tumor cells. PD-L2 is expressed only in non-haematopoietic cells, APCs and some tumors[57].

Soluble forms of PD1 and PDL1

Both PD-1 and PD-L1 exist in soluble forms: soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1). The sPD-L1 is expressed by tumor cells and mature DCs, whereas immature DCs, macrophages, monocytes and T lymphocytes are refractory to releasing sPD-L1. The sPD-L1 can bind membrane-bound PD-1, and inhibits the activation of T lymphocytes. sPD-L1 has been identified in the blood of cases with different malignancies, and also in serum and urine samples of BC cases[58].

Immune function of PD1 and its ligands

The adaptive immune cells eliminate any threat via the combined actions of $CD4^+$ T lymphocytes, $CD8^+$ T-lymphocytes and B-lymphocytes. For T lymphocytes, many regulatory mechanisms undergo activation during initial antigen-mediated activation. Early during activation, negative regulators counteract the activation program. PD1 is expressed during the activation of T-cells and counters positive costimulatory signals mediated through the T cell receptor and CD28. These 'co-inhibitory' receptors serve as immune checkpoints that effector T lymphocytes have to pass to exert their full function[59].

The PD1 expression promotes the regulation of initial activation of T-cells, fine-tuning of their function, T-cell tolerance and return to immune homeostasis. Excessive and sustained PD1 expression commonly occur in chronic infection and malignancy, in which PD1 pathway inhibition can improve the function of T cells and decrease viral load and tumor progression[60].

Treatment with PD1 pathway inhibitors has shown success in promoting durable antitumor immune response. So, FDA approved the monoclonal antibodies nivolumab (anti-PD1; Bristol-Myers Squibb, US), avelumab (anti-PDL1; EMD Serono, US) and durvalumab (anti-PDL1; AstraZeneca, UK) for use in cancer therapy. Nonetheless, the majority of cases do not have long-lasting remission, and many malignancies are totally refractory to PD1 pathway inhibitors[61].

Relation between PD1, PDL1, and Bladder cancer

For the survival and progression of cancer cells, they need to escape the immune response. So, cancer cells deregulate immune checkpoints. A key immune checkpoint is PD-L1 which binds to PD-1 to inhibit immune responses mediated by T-lymphocytes, thus cancer cells can evade immune surveillance[62]. Nevertheless, other studies demonstrated that there was no correlation between PD-L1 expression and clinico-pathological features, as well as association between PD-L1 overexpression and good clinical outcomes[63].

Relation between PDL1 and Epstein Barr Virus

Infected and malignant cells (including EBV-positive malignancies) often overexpress PD-L1 which binds to PD-1 on the surface of T lymphocytes to inhibit their activity. After the virus enters the host, it upregulates PD-L1 expression. This may occur through virus recognition by pattern recognition receptors (PRRs), including toll-like receptors (TLR)-3, TLR7, TLR8, and TLR9[64].

These PRRs also induce type I IFN pathway that regulates PD-L1[66]. Another pathway of PD-L1 upregulation is via viral replication. The latter is linked to the release of anti-inflammatory cytokines (e.g.IL-10) that result in PD-L1 upregulation. In addition, EBV is associated with PD-L1 overexpression on monocytes and increased ROS production. EBNA2 has an important role in PD-L1 induction in B-lymphocytes[66].

Rationale for Anti-PD-1/PDL1 Treatment in EBV

Enhanced PD-L1 expression observed in most of viruses that cause cancers has led to the assumption of whether anti-PD-1/PD-L1 immune checkpoint blockers might have a potential role in treating viral infection[67]. Anti-PD-1/PD-L1 immune checkpoint blockers have to

be utilized carefully as significant inhibition of PD-1/PD-L1 axis can cause autoimmunity. To avoid autoimmunity, it is recommended to utilize combination therapies to treat viral infections, while preventing autoimmunity[68].

Rationale for Anti-PD-1/PD-L1 in BC treatment

Many reports showed that PD-1/PD-L1 was overexpressed in tumor tissues from BC patients than in normal tissues[61]. PD-L1 tumor expression had an association with the higher tumour stage and grade, with significant expression in BCG-induced granulomata of BCG-un responders. These cases demonstrated PD-L1 expression upregulation in tumour and immune cells compared with BCG-responder cases, and RNA-sequencing showed a higher baseline PD-L1 among BCG- un-responders. Collectively, the PD-1/PD-L1 pathway appears to have an important role in the immune response of BC[69].

CONCLUSION

The potential involvement of EBV in BC pathogenesis and its relationship with PD-L1 expression highlight a complex interplay between viral infection, immune modulation, and tumor progression. While emerging evidence suggests that EBV may contribute to bladder cancer development through immune evasion mechanisms, further research is needed to establish a definitive link and understand the underlying molecular pathways. PD-L1 upregulation in EBV-associated tumors raises important questions regarding the role of immune checkpoints in BC and the potential for EBV-driven immune suppression. Investigating these interactions could provide important insights into novel diagnostic, prognostic, and therapeutic approaches, particularly in the context of immunotherapy. Future studies have to focus on elucidating the precise mechanisms of EBV-mediated oncogenesis in bladder cancer and exploring targeted treatment approaches for immune checkpoint inhibitors.

Abbreviations

Bladder cancer (BC), Epstein-Barr virus (EBV), Programmed cell death ligand 1 (PD-L1), Epstein-Barr nuclear antigens (EBNAs), Latent membrane proteins (LMP), Epstein-Barr virus encoded RNA (EBER), Programmed death 1 (PD1), Antigen presenting cells (APCs), Dendritic cells (DCs), Complement receptor (CR), cluster of differentiation (CD), Human leucocytic antigen (HLA), Immediate early (IE), Early (E), Late (L), Infectious mononucleosis (IM), Electron microscopy (EM), EBV-encoded small RNAs – In Situ Hybridization (EBER-ISH), Indirect fluorescent antibody (IFA), Anti-viral capsid antibody (Anti-VCA), Carcinoma-in-situ (CIS), Transurethral urethral resection of bladder tumor (TURBT), Non muscle invasive bladder cancer (NMIBC), Bacillus Calmette–Guérin (BCG), Muscle invasive bladder cancer (MIBC), Checkpoints inhibitors (ICIs), Cytotoxic T-lymphocyte associated protein-4 (CTLA-4), Natural Killer cells (NK), Soluble PD-1 (sPD-1), Soluble PD-L1 (sPD-L1), Pattern recognition receptors (PRRs), Toll-like receptors (TLRs), Interferon (IFN).

REFERENCES

1. Yasir S, Mahmood T, Abbas H. "Epstein–Barr Virus Infection Related to Low White Blood Cell Count in Cancer Patients Receiving Chemotherapy in Al-Najaf Governorate/Iraq." J Pure Appl Microbiol. 2020;14(2), 1609-1617.
2. Tselis A, Jenson HB, eds. Epstein-Barr Virus. Taylor and Francis, New York. 2006; https://doi. org/10.3109/9781420014280.
3. Moussa S, El-Sheshtawy W. Pathological Pattern of Urinary Bladder Cancer: Data from a Single Egyptian Institute. Research in Oncology. 2017; 13(1), 14-17.
4. Abe T, Shinohara N, Tada M, Harabayashi T, Sazawa A, Maruyama S, Moriuchi T, Takada K, Nonomura K. "Infiltration of Epstein-Barr virus-harboring lymphocytes occurs in a large subset of bladder cancers." International journal of urology. 2008; 15(5): 429-434.
5. Chuang CK, Liao SK. "Epstein-Barr virus infection in urothelial transitional cell carcinoma tissues." BJU international. 2004; 93(4): 495- 498.
6. Han Y, Liu D, Li, L. PD-1/PD-L1 pathway: current researches in cancer. American journal of cancer research. 2020; 10(3), 727.
7. Nagato T, Ohkuri T, Ohara, K, Hirata, Y, Kishibe, K, Komabayashi, Y, and Kobayashi, H. Programmed death-ligand 1 and its soluble form are highly expressed in nasal natural killer/T-cell lymphoma: a potential rationale for immunotherapy. Cancer Immunology, Immunotherapy. 2017; 66(7), 877-890.
8. Stenehjem DD, Tran D, Nkrumah MA, Gupta S. "PD1/PDL1 inhibitors for the treatment of advanced urothelial bladder cancer." OncoTargets and therapy. 2018; 11, 5973.
9. Li X, Zhang W. "Expression of PD-L1 in EBV-associated malignancies." International Immunopharmacology. 2021; 95, 107553.
10. Hoover, K, & Higginbotham, K. Epstein barr virus. 2020.
11. Siddell SG, Smith DB, Adriaenssens E, Alfenas-Zerbini P, Dutilh BE, Garcia ML, Junglen S, Krupovic M, Kuhn JH. Lambert AJ. Virus taxonomy and the role of the International Committee on Taxonomy of Viruses (ICTV). Journal of General Virology 2023; 104(5), 001840.

12. Alford C, Britt W, Whiteley R, Lopez C. Cytomegalovirus: The human herpesviruses. Roizman, Raven Press Ltd, New York. 1993; 227.
13. Damania, B Kenney SC, Raab-Traub N. Epstein-Barr virus: Biology and clinical disease. Cell. 2022; 185(20), 3652-3670.
14. Connolly SA, Jardetzky TS, Longnecker R. The structural basis of herpesvirus entry. Nature reviews Microbiology. 2021; 19(2), 110-121.
15. Cui X, Snapper CM. Epstein Barr virus: development of vaccines and immune cell therapy for EBV-associated diseases. Frontiers in immunology. 2021; 12, 734471.
16. Gärtner BC, Preiksaitis J. Epstein-Barr Virus. Manual of clinical microbiology. 2015; 1738-1753.
17. Odumade OA, Hogquist KA, Balfour Jr, HH. Progress and problems in understanding and managing primary Epstein-Barr virus infections. Clinical microbiology reviews. 2011; 24(1), 193-209.
18. Long HM, Meckiff BJ, Taylor GS. The T-cell response to Epstein-Barr virus–new tricks from an old dog. Frontiers in immunology. 2019; 10, 2193.
19. Aiyar A, Aras S, Washington A, Singh G, Luftig RB. Epstein-Barr Nuclear Antigen 1 modulates replication of oriP-plasmids by impeding replication and transcription fork migration through the family of repeats. Virology Journal. 2009; 6(1), 1-15.
20. Kelly GL, Long HM, Stylianou J, Thomas WA, Leese A, Bell AI, Bornkamm GW, Mautner J, Rickinson AB, Rowe M. An Epstein-Barr virus anti-apoptotic protein constitutively expressed in transformed cells and implicated in burkitt lymphomagenesis: the Wp/BHRF1 link. PLoS pathogens. 2009; 5(3), e1000341.
21. Thorley-Lawson DA, Hawkins JB, Tracy SI, Shapiro M. The pathogenesis of Epstein–Barr virus persistent infection. Current opinion in virology. 2013; 3(3), 227-232.
22. De Leo A, Calderon A, Lieberman PM. Control of viral latency by episome maintenance proteins. Trends in microbiology. 2020; 28(2), 150-162.
23. Murata T, Tsurumi T. Switching of EBV cycles between latent and lytic states. Reviews in medical virology. 2014; 24(3), 142-153.
24. Kenney SC, Mertz JE. Regulation of the latent-lytic switch in Epstein–Barr virus. Seminars in cancer biology. 2014.
25. Damania B, Kenney SC, Raab-Traub N. Epstein-Barr virus: Biology and clinical disease. Cell. 2022.
26. Wu Y, Ma S, Zhang L, Zu D, Gu F, Ding X, Zhang L. Clinical manifestations and laboratory results of 61 children with infectious mononucleosis. Journal of International Medical Research. 2020; 48(10), 0300060520924550.
27. Kimura H, Cohen JI. Chronic active Epstein–Barr virus disease. Frontiers in immunology. 2017; 8, 309967.
28. Yin H, Qu J, Peng Q, Gan R. Molecular mechanisms of EBV-driven cell cycle progression and oncogenesis. Medical microbiology and immunology. 2019; 208, 573-583.
29. Su ZY, Siak PY, Leong C-O, Cheah S-C. The role of Epstein–Barr virus in nasopharyngeal carcinoma. Frontiers in microbiology. 2023; 14, 1116143.
30. Allday MJ, Bazot Q, White RE. The EBNA3 family: two oncoproteins and a tumour suppressor that are central to the biology of EBV in B cells. Epstein Barr Virus Volume 2: One Herpes Virus: Many Diseases. 2015; 61-117.
31. Zhang Y, Guo W, Zhan Z, Bai O. Carcinogenic mechanisms of virus-associated lymphoma. Frontiers in immunology. 2024; 15, 1361009.
32. Abusalah MAH, Gan SH, Al-Hatamleh MA, Irekeola AA, Shueb RH, Yean Yean C. Recent advances in diagnostic approaches for epstein–barr virus. Pathogens. 2020; 9(3), 226.
33. Atefi A. Perspective Chapter: Epstein-Barr Virus–Emphasis on Diagnostic Approaches. 2023; In Viral Replication Cycle-From Pathogenesis and Immune Response to Diagnosis and Therapy. IntechOpen.
34. Nakatsuka S-i, Homma K, Aozasa K. When to use in situ hybridization for the detection of Epstein-Barr virus: a review of Epstein-Barr virus-associated lymphomas. Journal of Hematopathology. 2015; 8, 61-70.
35. Stuempfig ND, Seroy J. Monospot test. 2019.
36. Odumade OA, Hogquist KA, Balfour Jr, HH. Progress and problems in understanding and managing primary Epstein-Barr virus infections. Clinical microbiology reviews. 2011; 24(1), 193-209.
37. Bollard CM, Cohen JI. How I treat T-cell chronic active Epstein-Barr virus disease. Blood, The Journal of the American Society of Hematology. 2018; 131(26), 2899-2905.
38. Klemm J, Bekku K, Abufaraj M, Laukhtina E, Matsukawa A, Parizi MK, Karakiewicz PI, Shariat SF. Upper Tract Urothelial Carcinoma: A Narrative Review of Current Surveillance Strategies for Non-Metastatic Disease. Cancers. 2013; 16(1), 44.
39. Alifrangis C, McGovern U, Freeman A, Powles T, Linch M. Molecular and histopathology directed therapy for advanced bladder cancer. Nature Reviews Urology. 2019; 16(8), 465-483.
40. Dyrskjøt L, Hansel DE, Efstathiou JA, Knowles MA, Galsky MD, Teoh J, Theodorescu D. Bladder cancer. Nature Reviews Disease Primers. 2023; 9(1), 58.
41. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of

incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021; 71(3), 209-249.
42. Riegert-Johnson DL, Gleeson FC, Roberts M, Tholen K, Youngborg L, Bullock M, Boardman LA. Cancer and Lhermitte-Duclos disease are common in Cowden syndrome patients. Hereditary cancer in clinical practice. 2010; 8, 1-7.
43. Yan H, Ying Y, Xie H, Li J, Wang X, He L, Jin K, Tang J, Xu X, Zheng X. Secondhand smoking increases bladder cancer risk in nonsmoking population: a meta-analysis. Cancer management and research. 2018; 3781-3791.
44. Lobo N, Afferi L, Moschini M, Mostafid H, Porten S, Psutka SP, Gupta S, Smith AB, Williams SB, Lotan Y. Epidemiology, screening, and prevention of bladder cancer. European urology oncology. 2022; 5(6), 628-639.
45. Zaghloul MS, Zaghloul TM, Bishr MK, Baumann BC. Urinary schistosomiasis and the associated bladder cancer: update. Journal of the Egyptian National Cancer Institute. 2020; 32, 1-10.
46. Batista R, Vinagre N, Meireles S, Vinagre J, Prazeres H, Leão R, Máximo V, Soares P. Biomarkers for bladder cancer diagnosis and surveillance: a comprehensive review. Diagnostics. 2020; 10(1), 39.
47. Rai BP, Escrig JLD, Vale L, Kuusk T, Capoun O, Soukup V, Bruins HM, Yuan Y, Violette PD, Santesso N. Systematic review of the incidence of and risk factors for urothelial cancers and renal cell carcinoma among patients with haematuria. European urology. 2022; 82(2), 182-192.
48. Czech AK, Gronostaj K, Fronczek J, Frydrych J, Bezshapkin V, Przydacz M, Dudek P, Curylo L, Wiatr T, Gasowski J. Diagnostic accuracy of bimanual palpation in bladder cancer patients undergoing cystectomy: a prospective study. Urologic Oncology: Seminars and Original Investigations. 2023.
49. Nikas, I. P, Seide, S, Proctor, T, Kleinaki, Z, Kleinaki, M, & Reynolds, J. P. The paris system for reporting urinary cytology: A meta-analysis. Journal of Personalized Medicine. 2022, 12(2), 170.
50. Babjuk M, Böhle A, Burger M, Capoun O, Cohen D, Compérat EM, Hernández V, Kaasinen E, Palou J, Rouprêt M. EAU guidelines on non–muscle-invasive urothelial carcinoma of the bladder: update 2016. European urology. 2017; 71(3), 447-461.
51. Babjuk M, Burger M, Compérat EM, Gontero P, Mostafid AH, Palou J, van Rhijn BW, Rouprêt M, Shariat SF, Sylvester R. European association of urology guidelines on non-muscle-invasive bladder cancer (TaT1 and carcinoma in situ)-2019 update. European urology. 2019; 76(5), 639-657.
52. Milowsky MI, Rumble RB, Booth CM, Gilligan T, Eapen LJ, Hauke RJ, Boumansour P, Lee CT. Guideline on muscle-invasive and metastatic bladder cancer (European Association of Urology guideline): American Society of Clinical Oncology clinical practice guideline endorsement. Journal of clinical oncology. 2016; 34(16), 1945-1952.
53. Nadal, R, Bellmunt, J. Management of metastatic bladder cancer. Cancer treatment reviews. 2019; 76, 10-21.
54. Zhang Y, Zheng J. Functions of immune checkpoint molecules beyond immune evasion. Regulation of cancer immune checkpoints: molecular and cellular mechanisms and therapy. 2020; 201-226.
55. Taefehshokr N, Baradaran B, Baghbanzadeh A, Taefehshokr S. Promising approaches in cancer immunotherapy. Immunobiology. 2020; 225(2), 151875.
56. Schönrich G, Raftery MJ. The PD-1/PD-L1 axis and virus infections: a delicate balance. Frontiers in cellular and infection microbiology. 2019; 9, 207.
57. Shin S-J, Jeon YK, Kim P-J, Cho YM, Koh J, Chung DH, Go H. Clinicopathologic analysis of PD-L1 and PD-L2 expression in renal cell carcinoma: association with oncogenic proteins status. Annals of surgical oncology. 2016; 23, 694-702.
58. Tosev G, Wahafu W, Reimold P, Damgov I, Schwab C, Aksoy C, Kaczorowski A, Stenzinger A, Nyarangi-Dix J, Hohenfellner M. Detection of PD-L1 in the urine of patients with urothelial carcinoma of the bladder. Scientific reports. 2021; 11(1), 14244.
59. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nature Reviews Immunology. 2018; 18(3), 153-167.
60. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends in immunology. 2015; 36(4), 265-276.
61. Day D, Hansen AR. Immune-related adverse events associated with immune checkpoint inhibitors. BioDrugs. 2016; 30(6), 571-584.
62. Boussiotis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. New England Journal of Medicine. 2016; 375(18), 1767-1778.
63. Breyer J, Wirtz RM, Otto W, Erben P, Worst TS, Stoehr R, Eckstein M, Denzinger S, Burger M, Hartmann A. High PDL1 mRNA expression predicts better survival of stage pT1 non-muscle-invasive bladder cancer (NMIBC) patients. Cancer Immunology, Immunotherapy. 2018; 67, 403-412.
64. Satou A, Nakamura S. EBV-positive B-cell lymphomas and lymphoproliferative disorders: Review from the perspective of immune escape and immunodeficiency. Cancer Medicine. 2021; 10(19), 6777-6785.
65. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010; 140(6), 805-820.

66. Yanagi Y, Okuno Y, Narita Y, Al Masud HA, Watanabe T, Sato Y, Kanda T, Kimura H, Murata T. RNAseq analysis identifies involvement of EBNA2 in PD-L1 induction during Epstein-Barr virus infection of primary B cells. Virology. 2021; 557, 44-54.
67. Rao M, Valentini D, Dodoo E, Zumla A, Maeurer M. Anti-PD-1/PD-L1 therapy for infectious diseases: learning from the cancer paradigm. International Journal of Infectious Diseases. 2017; 56, 221-228.
68. Balsitis S, Gali V, Mason PJ, Chaniewski S, Levine SM, Wichroski MJ, Feulner M, Song Y, Granaldi K, Loy JK. Safety and efficacy of anti-PD-L1 therapy in the woodchuck model of HBV infection. PloS one. 2018; 13(2), e0190058.
69. de Jong FC, Rutten VC, Zuiverloon TC, Theodorescu D. Improving anti-PD-1/PD-L1 therapy for localized bladder cancer. International Journal of Molecular Sciences. 2021; 22(6), 2800.