香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Insights into EBV and HPV co-infection as a contributing factor in cancers

Student: Zeyuan WANG, Kevin

2nd Year PhD Student

Supervisor: Prof. Zigui CHEN

Date: 11. 12. 2024

Outline

1. Background of oncovirus
2. Introduction of HPV and EBV
3. EBV/HPV-related diseases
4. Carcinogenetic mechanism of EBV/HPV
5. Prevention and therapy

1 Background of oncovirus

- 15.4% of cancers are attributable to infections. 9.9% are linked to viruses. {WHO}
- 11 pathogens have been classified as carcinogenic agents (Group 1) in humans. {IARC}

Group 1 agent	Cancers for which there is sufficient evidence in humans	Other sites with limited evidence in humans	Established mechanistic events
Epstein–Barr virus (EBV)	Nasopharyngeal carcinoma, Burkitt's lymphoma, immune-suppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type), Hodgkin's lymphoma	Gastric carcinoma,* lympho-epithelioma-like carcinoma*	Cell proliferation, inhibition of apoptosis, genomic instability, cell migration
Hepatitis B virus (HBV)	Hepatocellular carcinoma	Cholangiocarcinoma,* non-Hodgkin lymphoma*	Inflammation, liver cirrhosis, chronic hepatitis
Hepatitis C virus (HCV)	Hepatocellular carcinoma, non-Hodgkin lymphoma*	Cholangiocarcinoma*	Inflammation, liver cirrhosis, liver fibrosis
Kaposi's sarcoma herpes virus (KSHV)	Kaposi's sarcoma,* primary effusion lymphoma*	multicentric Castleman's disease*	Cell proliferation, inhibition of apoptosis, genomic instability, cell migration
Human immunodeficiency virus, type 1 (HIV-1)	Kaposi's sarcoma, non-Hodgkin lymphoma, Hodgkin's lymphoma,* cancer of the cervix,* anus,* conjunctiva*	Cancer of the vulva,* vagina,* penis,* non-melanoma skin cancer,* hepatocellular carcinoma*	Immunosuppression (indirect action)
Human papillomavirus type 16 (HPV-16)†	Carcinoma of the cervix, vulva, vagina, penis, anus, oral cavity, and oropharynx and tonsil	Cancer of the larynx	Immortalisation, genomic instability, inhibition of DNA damage response, anti-apoptotic activity
Human T-cell lymphotrophic virus, type-1 (HTLV-1)	Adult T-cell leukaemia and lymphoma	..	Immortalisation and transformation of T cells
Helicobacter pylori	Non-cardia gastric carcinoma, low-grade B-cell mucosa-associated lymphoid tissue (MALT) gastric lymphoma*	..	Inflammation, oxidative stress, altered cellular turn-over and gene expression, methylation, mutation
Clonorchis sinensis	Cholangiocarcinoma*	..	..
Opisthorchis viverrini	Cholangiocarcinoma	..	Inflammation, oxidative stress, cell proliferation
Schistosoma haematobium	Urinary bladder cancer	..	Inflammation, oxidative stress

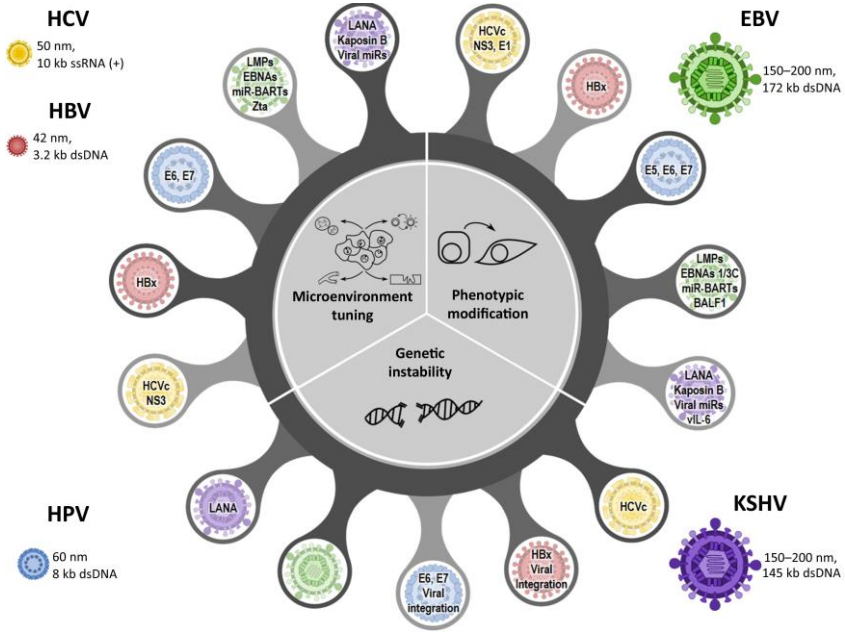
*Newly identified link between virus and cancer. †For other types, see table 2.

Table 1: **Biological agents assessed by the IARC Monograph Working Group**

{Hu M, Front Microbiol. 2024}
{Bouvard, V., Lancet Oncol. 2009}

1.1 Mechanism of cancer progression by oncoviruses

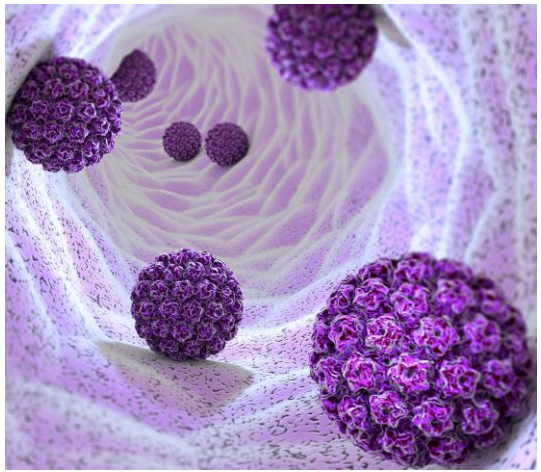
- Viral infection can lead to uncontrolled cell proliferation and transformation.
- Genetic instability, cell phenotypic modifications, and microenvironmental tuning.



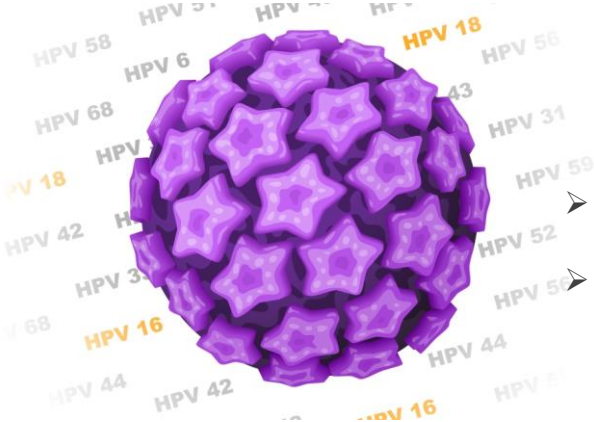
{Müller-Coan BG, Trends Cancer. 2018. }

2 Introduction of HPV and EBV

- Human papillomaviruses (HPVs) belong to the family of *Papillomaviridae*.
- HPVs infect the skin and mucosa, are the most common sexually transmitted viruses.
- Most HPV infections are benign, persistent infection with HR-HPV may cause cancer.



{umiamihealth.org}



{thewellproject.org}

- 13 High-Risk/oncogenic types.
- Low-Risk/non-oncogenic types.

香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

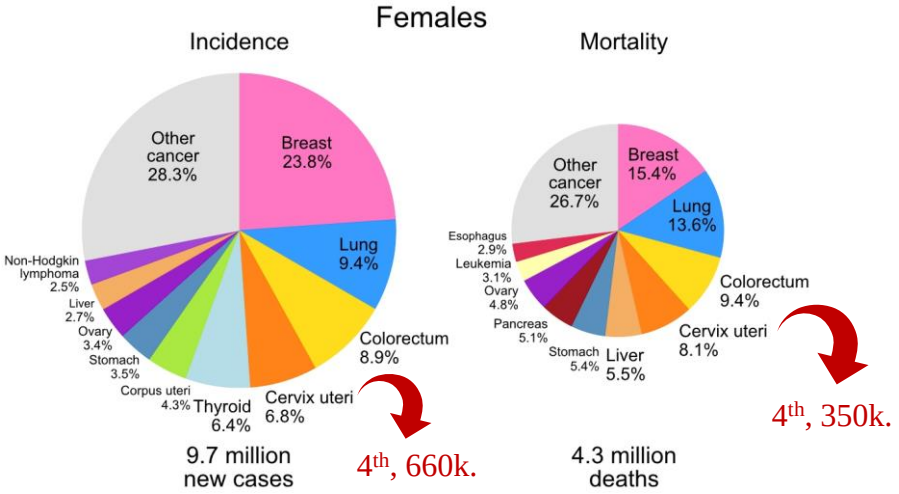
{Martinelli M, Int J Environ Res Public Health, 2019.}
{McBride, Nat Rev Microbiol, 2022. }

2.1 HPV-related diseases

- Persistent HR-HPV infection is strongly associated with several types of cancer.
- Cervical cancer is the 4th most common cancer in women.

the GLOBOCAN 2022

- Cervical cancer (99.7%);
- Head and neck squamous cell carcinomas (60%);
- Anal cancer (93%);
- Vulvar cancer (69%);
- Vaginal cancer (75%);
- Penile cancers (47%).

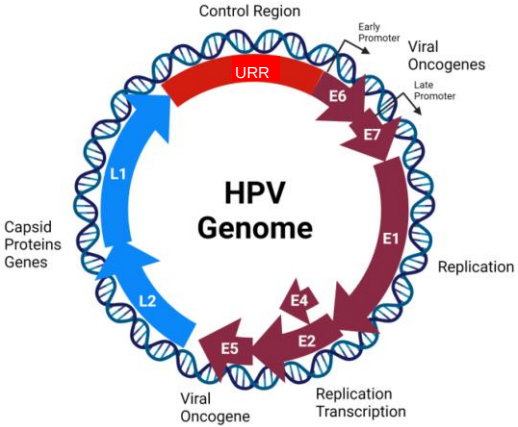


Distribution of incidence and mortality for the top10 most common cancers in 2022 for females.

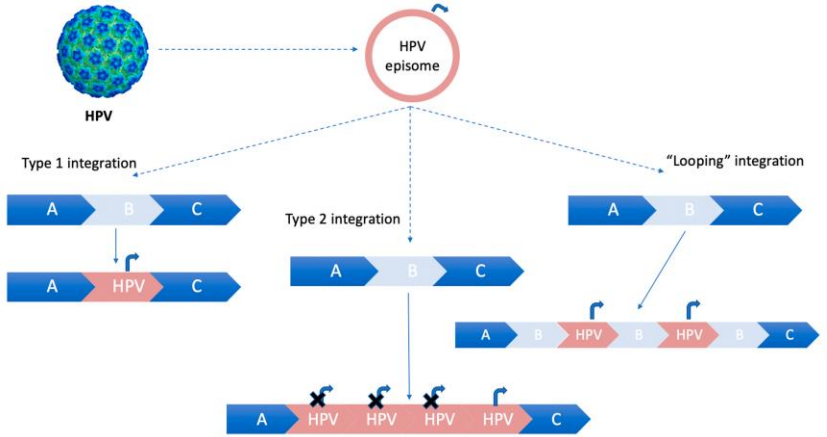
{Bray F, CA Cancer J Clin. 2024}
{Hausen, Virology, 2009. }

2.2 HPV genome

- Circular double-stranded DNA genomes, approximately 8 kb in size.
- Core proteins: genome replication (E1 and E2), assembly (L1 and L2).
- Accessory proteins (E4, E5, E6, and E7) vary in expression time and functional properties.
- HPV-DNA can integrate into host chromosomes.



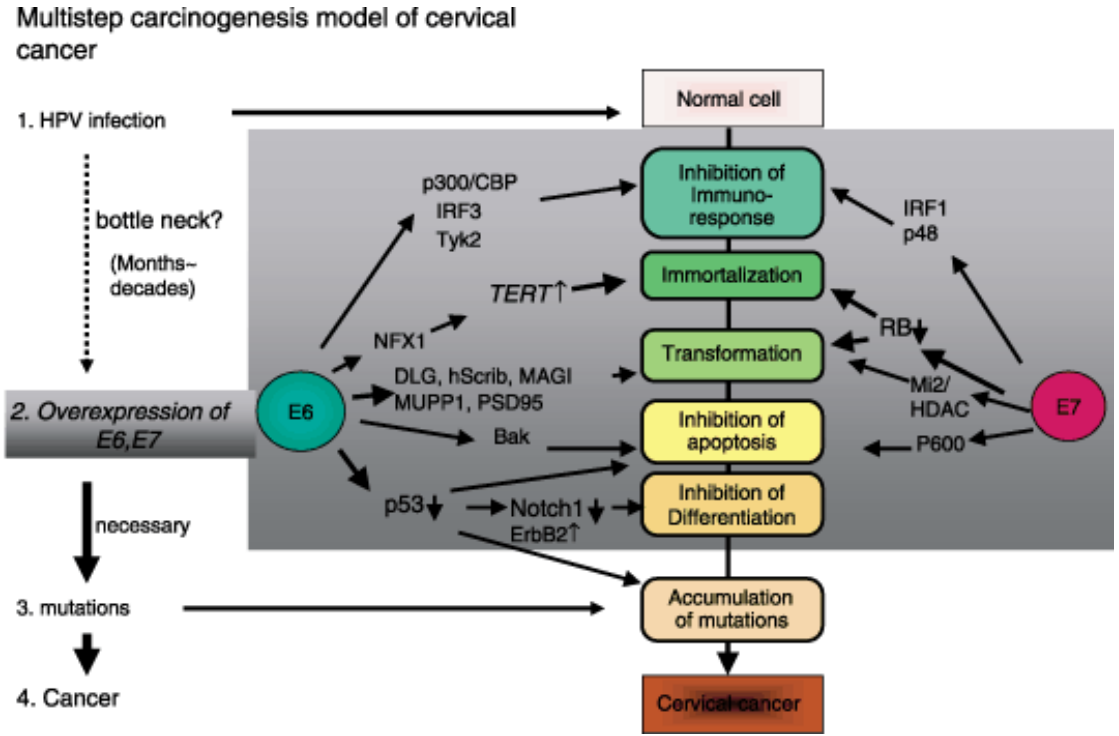
HPV genome structure.



HPV integration into the host genome.

{Scudellari, Nature, 2013. }
{Blanco, Biology (Basel), 2021}

2.3 HPV carcinogenetic mechanism

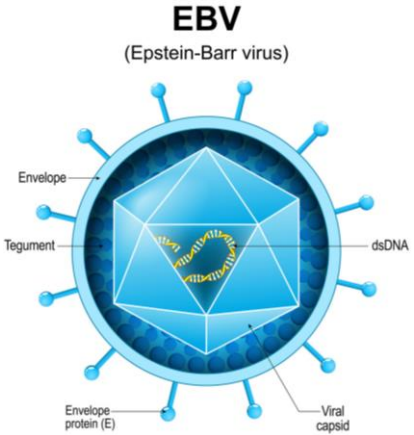


Schematic flowchart of HPV carcinogenesis model of cervical cancer.

{Narisawa-Saito M,. Cancer Sci. 2007. }

3 Introduction of HPV and EBV

- Epstein-Barr virus (EBV), also known as human herpesvirus 4 (HHV-4).
- First discovered in 1964, causing lymphoma and epithelial carcinoma, including nasopharyngeal carcinoma (NPC), gastric cancer and breast cancer.
- EBV infects over 95% of the world's population, establishes life-long latent infection in B lymphocytes.

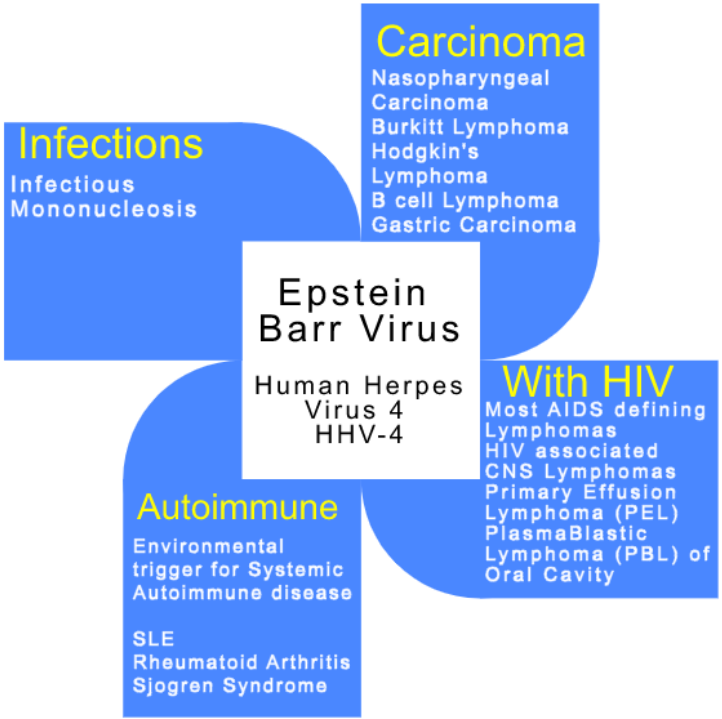


Acute	Rash
	Airway obstruction
	Splenic (e.g. splenomegaly)
	Lemierre's disease
Delayed	Chronic active EBV infection
	Multiple sclerosis (MS)
	Oral hairy leukoplakia
	Lymphoproliferative disorders
	Hemophagocytic lymphohistiocytosis
	Lymphomatoid granulomatosis
	X-linked lymphoproliferative disease
	Post-transplant lymphoproliferative disease
Malignancy	Burkitt's lymphoma
	Hodgkin's lymphoma
	Nasopharyngeal carcinoma
	Gastric carcinoma
	T cell lymphoma

The complications of EBV.

{Damania B, Cell, 2022. }
{Ebrahimi. F, Frontiers in Virology, 2024}

3.1 EBV-related diseases



{http://notesmedicalstudent.blogspot.com}

- Infectious mononucleosis and glandular fever in the adolescents.
- Various Systemic Autoimmune disease.
- Lymphoproliferative diseases.
- First known oncovirus to cause cancers in human.

3.1.1 EBV-related NPC

Characteristic	n (%)	Pre-DNA, copies/mL		P
		< 1500	≥ 1500	
Age (years)				0.892
< 60	912 (88.0)	346	566	
≥ 60	124 (12.0)	51	73	
Gender				0.051
Male	785 (75.8)	287	498	
Female	251 (24.2)	109	142	
Histology				0.943
WHO II	59 (5.7)	21	38	
WHO III	977 (94.3)	375	602	
T stage[a)]				0.069
T1	42 (4.1)	20	22	
T2	56 (5.4)	18	38	
T3	736 (71.0)	289	447	
T4	202 (19.5)	69	133	
N stage[a)]				0.162
N0	122 (11.8)	60	62	
N1	580 (56.0)	228	352	
N2	222 (21.4)	72	150	
N3	112 (10.8)	36	76	
Clinical stage[a)]				0.071
III	762 (73.6)	298	464	
IVA-B	274 (26.4)	98	176	
Treatment method				0.357
IMRT alone	64 (6.2)	33	31	
CCRT	630 (60.8)	248	382	
NACT + CCRT	342 (33.0)	115	227	

- 1036 locoregionally advanced nasopharyngeal carcinoma (LA-NPC) patients were enrolled at the Cancer Center of Sun Yat-sen University in Guangzhou.
- Patients with stage III, IVA, or IVB cancer and with high EBV copy number in blood (>1500 viral copies/ml) showed poorer outcomes compared with patients with low levels of EBV viremia.

{Jin YN, J Cancer. 2017. }

3.1.2 EBV-related NPC

- A study aimed to investigate EBV-oncoprotein LMP1 and LMP2 expression in relation to therapy outcome and 24-months survival in a series of Indonesian NPC patients (56 NPC patients).
- High LMP expression was associated with poor outcome.

Table 1. Characteristic of Subjects

	Response of therapy(-)	Response of therapy(+)	P
Age			
11-20	2	1	0.93
21-30	2	2	
31-40	3	2	
41-50	11	11	
51-60	6	5	
61-70	4	5	
> 70	1	1	
Sex			
Male	19	21	0.55
Female	9	7	
Stage			
III	14	16	0.55
IVA	3	3	
IVB	11	9	
PA			
WHO II	2	-	0.30
WHO III	26	28	
Therapi			
Protocol A	12	8	0.26
Protocol B	26	20	

Table 4. Bivariate Analysis of Protein Expression Toward 24-month Survival Rate

Variable		Outcome: Death n (%)	Outcome: Survive n (%)	OR (95 % IK)	P
Sex	Male	11 (84,6%)	28 (65,1%)	2,95 (0,58 - 15,07)	0,180
	Female	2 (15,4%)	15 (34,9%)		
Pathologic	WHO III	13 (100,0%)	42 (97,7%)	0,65 (0,06 - 7,74)	0,579
	WHO II	0	1 (2,3%)		
LMP 1	≥ 7,2	11 (84,6%)	16 (37,2%)	9,28 (1,82 - 47,30)	0,003*
	< 7,2	2 (15,4%)	27 (62,8%)		
LMP 2	≥ 2,7	10 (76,9%)	17 (39,5%)	5,10 (1,22 - 21,25)	0,018*
	< 2,7	3 (23,1%)	26 (60,5%)		

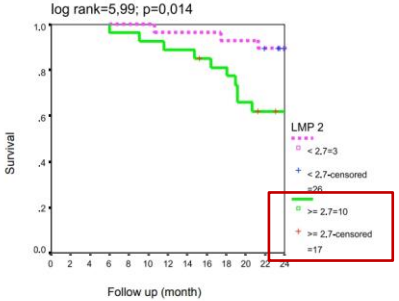


Figure 1. Difference in 24-month Survival between LMP2 > 2.7 and LMP2 < 2.7

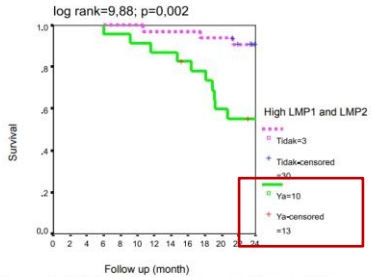
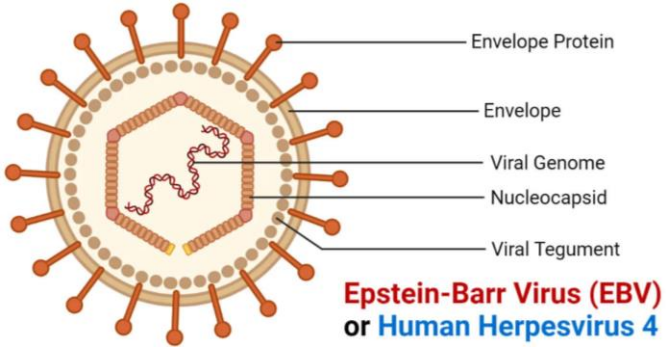


Figure 2. Difference in 24-month Survival between NPC Expressing LMP1>7.2 and LMP2>2.7 Compared to those Expressing LMP1<7.2 and LMP2<2.7

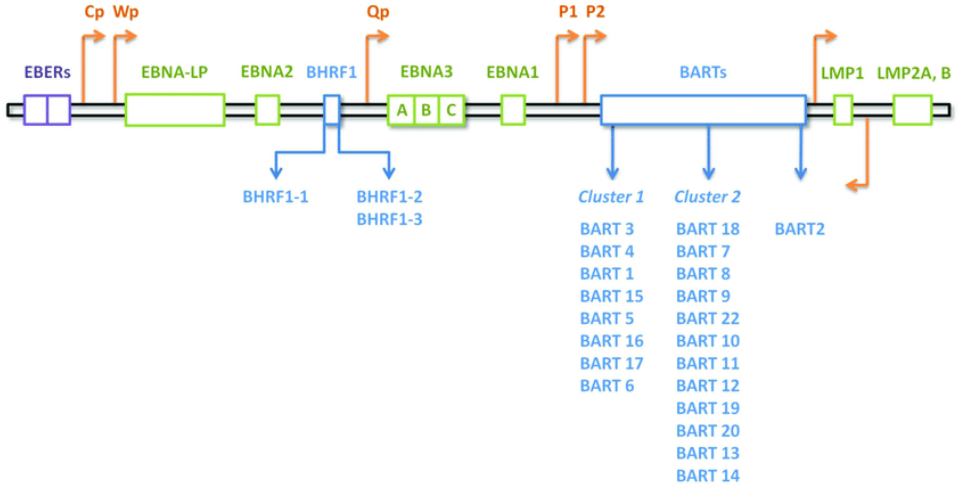
{Hariwiyanto B, Asian Pac J Cancer Prev. 2010.}

3.2 EBV genome

- EBV contains a linear double-stranded linear DNA genome, approximately 172k bp.
- EBV is enclosed by a capsid, composed of enzymes and proteins involved in viral replication.
- EBV encodes more than 85 genes and 44 mature miRNAs.



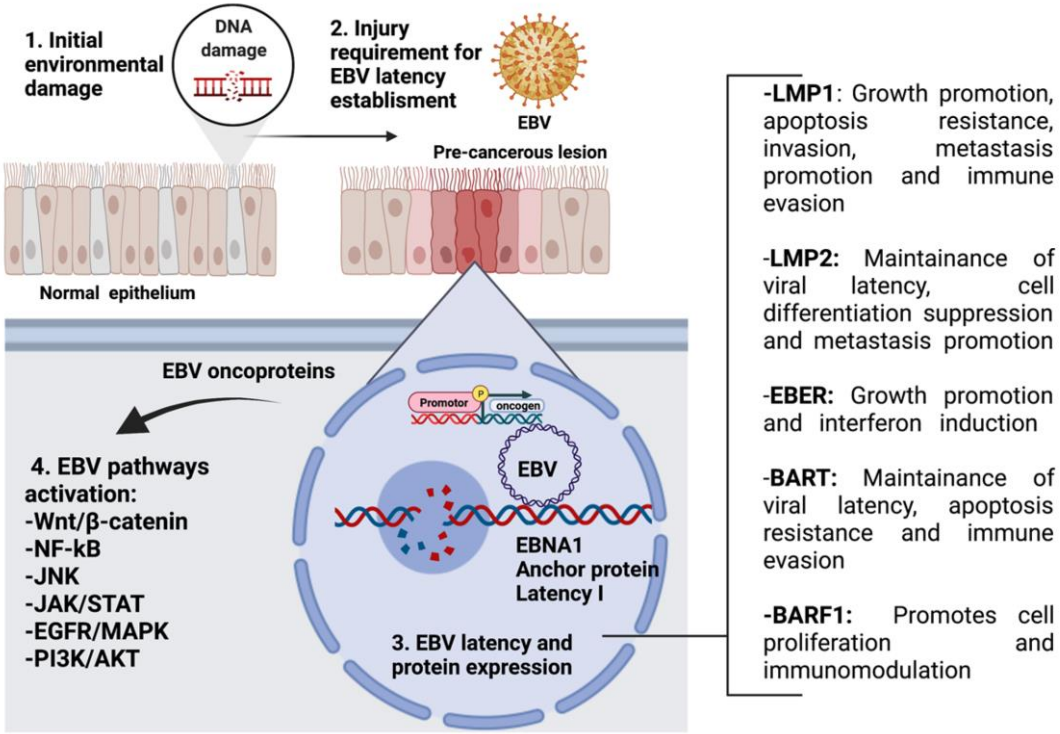
EBV genome structure.



The structure of the EBV genome encoding latent products.

{Osorio JC, Pathogens. 2022. }

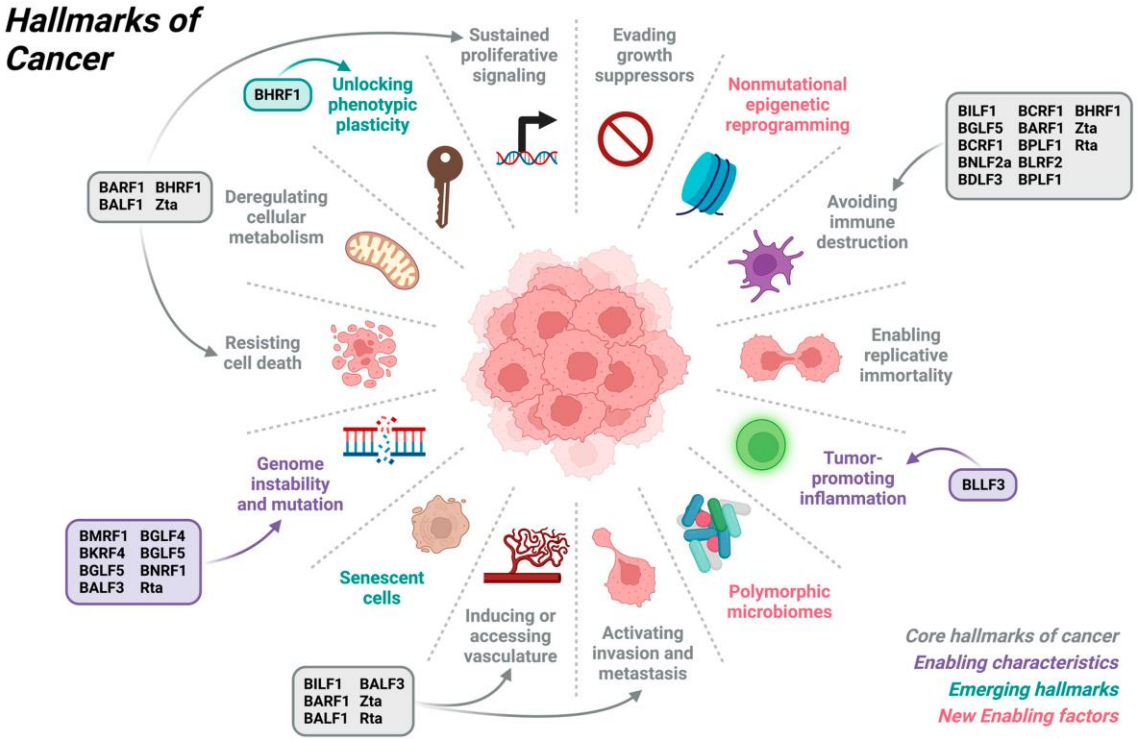
3.3 EBV carcinogenetic mechanism



EBV proteins in epithelial tumors and functions in cancer progression.

{Osorio JC, Pathogens. 2022. }

3.3 EBV carcinogenetic mechanism



Contribution of EBV lytic proteins to hallmarks of cancers.

4 HPV/EBV co-infection

- Most HPV infections resolve spontaneously by the immune system.
- The risk of developing cancer in HR-HPVs infection is low. (e.g., only 10% of HPV +ve women will develop cervical cancer.) {cdc.gov/cancer/hpv}

HPV

Cancer

- Co-infection with other <u>infectious agents</u> as additional risk factors may reduce the host ability to clear HPV and increase the risk of HPV infection-related malignancies.
- EBV Epstein–Barr virus etc.
  HSV Herpes simplex virus
  HIV Human immunodeficiency virus
  CMV Cytomegalovirus

{Akbari E, J Med Virol, 2023}

4.1 HPV/EBV co-infection related diseases

- Many studies have explored the prevalence of HPV/EBV and the association between HPV/EBV co-infection and clinicopathological characteristics or cancer progression.
- 3 cohort studies regard colorectal cancer, prostate cancer, head and neck cancer.
- Insights into EBV/HPV co-infection as a contributing factor in cancers.

Table 1. Comparison between HPV and EBV epithelial cell entry.

Biological Process	Human Papillomavirus	Epstein–Barr Virus	References
Route of entry	Direct epithelial contact, apical entry (microlesions)	Salivary transmission, apical, basolateral, or basal entry	[21,33]
Tropism	Epithelial cells (mucosal or cutaneous)	Epithelial cells (mucosal), B cells, T cells, NK cells	[33,34]
Entry mechanism	Endocytosis	Membrane fusion	[35,36]
Receptors	Entry receptor complex, HSPGs, integrins	EphA2	[24,37]

HSPGs, heparan sulphate proteoglycans; EphA2, ephrin receptor tyrosine kinase A2.

4.1.1 HPV/EBV co-infection related colorectal cancer

- 100 formalin-fixed paraffin-embedded (FFPE) tissue samples diagnosed during 2018~2021 in Qatar were included.
- This study revealed a 17% co-infection rate of HPVs and EBV in CRC cases and a significant correlation only between HPV45 and EBV.
- HPV/EBV co-infection didn't significantly associate with clinicopathological characteristics, However, a coinfection of two or more HR-HPVs is a powerful predictor, and EBV infection confounds this association resulting in 4.39 higher odds of developing CRC compared to individuals without this coinfection.

Status of Presence and Co-presence of HPV and EBV in Colorectal Cancer Patients from Qatar

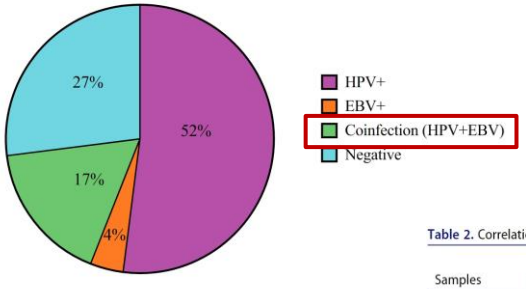


Table 3. Coinfection of various HPV subtypes with EBV.

Coinfection of HPV Subtypes with EBV	
HPV Subtypes	Cases
Single HPV infection + EBV ($n = 7$)	
HPV16 + EBV	1
HPV18 + EBV	4
HPV52 + EBV	2
Double HPV infections + EBV ($n = 4$)	
HPV18 & HPV52 + EBV	1
HPV35 & HPV52 + EBV	1
HPV52 & HPV45 + EBV	2
Triple HPV infections + EBV ($n = 1$)	
HPV18, HPV31 & HPV52 + EBV	1
Quadruple HPV infections + EBV ($n = 3$)	
HPV18, HPV31, HPV45 & HPV52 + EBV	2
HPV35, HPV45, HPV52 & HPV59 + EBV	1
Quintuple HPV infections + EBV ($n = 2$)	
HPV18, HPV31, HPV45, HPV51 & HPV52 + EBV	1
HPV18, HPV31, HPV45, HPV52 & HPV59 + EBV	1

Table 2. Correlation of EBV and HPV subtypes in colorectal cancer patients from Qatar.

		High-Risk HPV Subtypes							
Samples	No. of Cases	HPV16	HPV18	HPV31	HPV 35	HPV45	HPV51	HPV52	HPV59
EBV (-)	79	3	26	9	3	7	5	29	15
EBV (+)	21	1	10	5	2	7	1	12	2
Total	100	4	36	14	5	14	6	41	17
***p*-value**		**.84**	**.21**	**.14**	**.28**	**.004**	**.78**	**.091**	**.3**

Table 4. Association of advanced* colorectal cancer with HPV, two or more subtypes of HPV and EBV coinfection. ($n = 100$).

Variables	Odds ratio	95% confidence interval	*p*-value
Unadjusted (EBV)	1.05	0.39–2.82	.92
Unadjusted (Coinfection of HPV & EBV)	0.99	0.36–2.7	.7
Coinfection of HPV & EBV#	0.82	0.28–2.47	.11
Unadjusted (Coinfection with two or subtypes of HPV & EBV)	1.56	0.38–6.41	.53
Coinfection with two or subtypes of HPV & EBV#	1.01	0.22–4.61	.1

#Adjusted for age, gender, and histological Grade.

Table 5. Association of advanced colorectal cancer with a coinfection of HPV high-risk subtype as the main predictor and EBV. ($n = 100$).

Variables	Odds ratio	95% confidence interval	*p*-value
Unadjusted (HPV as the main predictor and EBV as a confounder)	3.96	1.50–10.47	.013
HPV as the main predictor and EBV as confounder#	4.39	1.6–11.98	.004

#adjusted for Age, Gender, and Histological Grade.

{Fernandes Q, Hum Vaccin Immunother. 2023}

4.1.2 HPV/EBV co-infection related prostate cancer

- This study included 67 patients with PCa and 40 healthy contro
  coinfection was 14.9% in patients and 7.5% in controls.
- No significant relationship was observed between PCa and HP
  of HPV genome integration was found in the HPV/EBV-co
- Expression levels of inflammatory factors, anti-apoptotic
  higher while tumor-suppressor proteins and E-cadherin sig

Table 1 Comparison of the characteristics of research units between prostate cancer and control groups

Characteristics		Prostate Cancer (67)	Control (40)	P	OR (95% CI)
Age (Year)		52.7 ± 12.2	55.6 ± 9.9	0.19	0.95 (0.9–1.02)
HPV positive samples	Presence	31.3% (*n* = 21)	15% (n = 6)	0.060	1.84 (0.08–0.6)
EBV positive samples	Presence	49.3% (*n* = 33)	40% (*n* = 16)	0.353	1.06 (0.8–1.39)
Mono HPV-infection		16.4% (11)	7.5% (n = 3)	0.289	1.06 (0.26–6.5)
Co-infection (EBV&HPV)		14.9% (n = 10)	7.5% (n = 3)	0.314	2.9 (0.18–45.2)
Mono EBV-infection		34.3% (*n* = 23)	42.5% (*n* = 17)	0.48	0.58 (0.17–2.6)
Non-EBV and Non-HPV samples		34.3% (n = 23)	42.5% (n = 17)	0.48	NA

Table 2 The HPV genome physical state in the studied groups

Status	PCa group	Controls group	Mono HPV infected PCa group	Co-infected PCa group	Mono HPV infected (%) Control group	Co-infected Control group
Fully integrated state (E2/E6 = 0)	9/20 (45%)	1/5 (20%)	1/11 (9.09%)	8/10 (80%)	0	1/3 (33.33%)
	P: 0.86		P: 0.0009		P: NA	
Episomal state (E2/E6 = 1)	2/20 (10%)	1/5 (20%)	2/11 (18.18%)	0	0	1/3 (33.33%)
	P: 0.62		P: NA		P: NA	
Mixed state (E2/E6 = > 0 to < 1)	10/20 (50%)	4/5 (80%)	8/11 (72.72%)	2/10 (20%)	3/3 (100%)	1/3 (33.33%)
	P:0.74		P: 0.003		P: 0.002	

NA Not applicable

Table 6 Comparison of cellular factors levels between the co-infection and mono-infection groups

Cellular Factors	Co-infected PCa (a) vs not co-infected PCa (b)	Co-infected Positive control (c) vs not co-infected control (d)	Co-infected PCa (a) vs HPV-mono-infected PCa (e)	Co-infected PCa (a) vs EBV-mono-infected PCa (f)	Co-infected PCa (a) and non-HPV/non-EBV PCa (g)
NF-κB	a: 26.9 ± 3.1	c: 21.6 ± 2.5	a: 26.9 ± 3.1	a: 26.9 ± 3.1	a: 26.9 ± 3.1
	b: 17.8 ± 7.1	d: 8.2 ± 6	e: 20.3 ± 7.2	f: 18.4 ± 3.2	g: 12 ± 8
	P* (0.0004)	P+ (0.004)	P$ (ns)	P^ (ns)	P# (0.0001)
TNF-α	a: 25.5 ± 2.7	c: 19.5 ± 3.1	a: 25.5 ± 2.7	a: 25.5 ± 2.7	a: 25.5 ± 2.7
	b: 15.2 ± 6.8	d: 10.6 ± 6.3	e: 19 ± 7.6	f: 16.2 ± 4.8	g: 10.2 ± 6.3
	P* (0.001)	P+ (ns)	P$ (ns)	P^ (0.04)	P# (0.0001)
IL-1	a: 18.4 ± 3.6	c: 18.3 ± 3.5	a: 18.4 ± 3.6	a: 18.4 ± 3.6	a: 18.4 ± 3.6
	b: 14.2 ± 4.4	d: 6.7 ± 4.2	e: 16.1 ± 4.6	f: 12.5 ± 4.2	g: 7.1 ± 4
	P* (ns)	P+ (0.004)	P$ (ns)	P^ (ns)	P# (0.0001)

Table 6 Comparison of cellular factors levels between the co-infection and mono-infection groups *(Continued)*

Cellular Factors	Co-infected PCa (a) vs not co-infected PCa (b)	Co-infected Positive control (c) vs not co-infected control (d)	Co-infected PCa (a) vs HPV-mono-infected PCa (e)	Co-infected PCa (a) vs EBV-mono-infected PCa (f)	Co-infected PCa (a) and non-HPV/non-EBV PCa (g)
Bcl-2	a: 14.1 ± 1.2	c: 15.4 ± 1.3	a: 14.1 ± 1.2	a: 14.1 ± 1.2	a: 14.1 ± 1.2
	b: 8.8 ± 3	d: 5 ± 2.6	e: 13.5 ± 1	f: 8 ± 2.1	g: 7.2 ± 1.9
	P* (0.005)	P+ (0.0004)	P$ (ns)	P^ (0.005)	P# (0.001)
CD44	a: 10.6 ± 5.4	c: 9.3 ± 1.5	a: 10.6 ± 5.4	a: 10.6 ± 5.4	a: 10.6 ± 5.4
	b: 7.2 ± 4.8	d: 3.5 ± 1.7	e: 3.7 ± 1.4	f: 10.8 ± 4.4	g: 3.9 ± 2
	P* (ns)	P+ (ns)	P$ (0.03)	P^ (ns)	P# (0.01)
TWIST	a: 26.8 ± 6.7	c: 23 ± 2	a: 26.8 ± 6.7	a: 26.8 ± 6.7	a: 26.8 ± 6.7
	b: 6.8 ± 10.1	d: 4.6 ± 4.9	e: 25.6 ± 9.9	f: 2.5 ± 1.3	g: 2.3 ± 1.4
	P* (< 0.0001)	P+ (0.009)	P$ (ns)	P^ (< 0.0001)	P# (< 0.0001)
E-cad	a: 2.7 ± 0.6	c: 2.6 ± 0.5	a: 2.7 ± 0.6	a: 2.7 ± 0.6	a: 2.7 ± 0.6
	b: 15.1 ± 7.7	d: 14.6 ± 4.6	e: 4.5 ± 3	f: 18 ± 5.8	g: 16.8 ± 6.9
	P* (< 0.0001)	P+ (0.007)	P$ (ns)	P^ (< 0.0001)	P# (< 0.0001)
N-cad	a: 25.9 ± 6.8	c: 25.3 ± 7.3	a: 25.9 ± 6.8	a: 25.9 ± 6.8	a: 25.9 ± 6.8
	b: 7.6 ± 9.8	d: 3.8 ± 5.5	e: 25.6 ± 9.7	f: 3.4 ± 1.8	g: 3.4 ± 2.5
	P* (0.001)	P+ (0.02)	P$ (ns)	P^ (0.0004)	P# (0.0003)
PTPN13	a: 6.2 ± 7.6	c:2 ± 1	a: 6.2 ± 7.6	a: 6.2 ± 7.6	a: 6.2 ± 7.6
	b: 10.5 ± 5.3	d: 14.1 ± 5.8	e: 3.6 ± 3.6	f: 12.7 ± 4.7	g: 12.5 ± 4
	P* (ns)	P+ (0.002)	P$ (ns)	P^ (0.03)	P# (0.03)
SLUG	a: 3.5 ± 1.6	c: 3.2 ± 1.4	a: 3.5 ± 1.6	a: 3.5 ± 1.6	a: 3.5 ± 1.6
	b: 1.8 ± 1.8	d: 0.96 ± 2.8	e: 4.3 ± 1.1	f: 0.9 ± 1.4	g: 1 ± 1.1
	P* (ns)	P+ (ns)	P$ (ns)	P^ (0.004)	P# (0.01)

Geometric Mean ± Standard Deviation, *: comparison between group a versus group b, +: comparison between group c versus group d, $: comparison between group e versus group f, ^: $: comparison between group g versus group h, FDR correction for multiple comparisons by Benjamini-Hochberg method

4.1.3 HPV/EBV co-infection related head and neck cancer

- This study included 98 HNSCC FFPE tissues from Bosnian patients.
- HPVs and EBV are co-present in (34/98) 34.7% of the oral and larynx SCC samples.
- 37.5% of oral SCCs are both HPV +ve and EBV +ve, with significant association between HR-HPVs and EBV.
- This data revealed that the co-presence of HPV and EBV is significantly correlated with advanced tumor stage.

Table 2 Prevalence of high-risk HPV types in relation to EBV status in HNSCC cohort (n = 98)

Samples	No. of cases	High-risk HPV types								
		16	18	31	33	35	45	51	52	58
EBV (+)	68	10	31	8	0	0	20	7	10	27
EBV (−)	30	11	24	1	0	3	7	2	4	20
Total	98	21	55	9	0	3	27	9	14	47
p-value		0.03*	0.003**	0.34	N/A	N/A	0.04*	0.85	0.89	0.02*

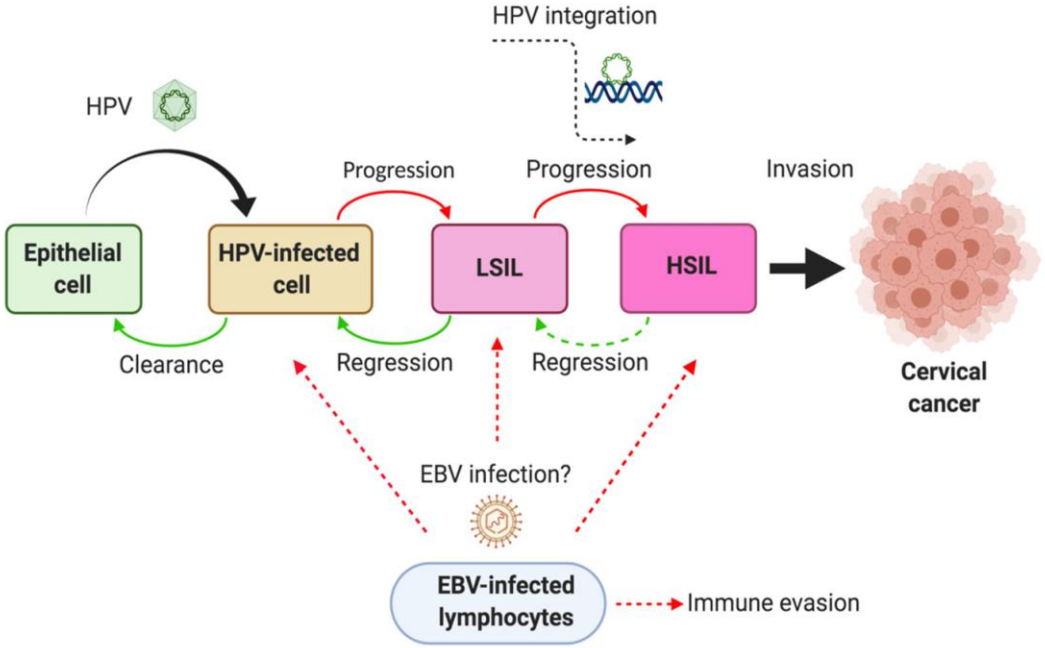
Significant *p*-values are denoted by asterisk (*)

Table 4 Correlation of clinicopathological characteristics with EBV/HPV positivity

Stages	HPV ±/EBV ± (%)	HPV +/EBV + (%)	p-value
pT1	16 (29)	2 (3.6)	p = 0.035*
pT2	14 (25.4)	5 (9)	
pT3	4 (7.2)	5 (9)	
pT4	4 (7.2)	5 (9)	
Total	38 (69)	17 (30.9)	

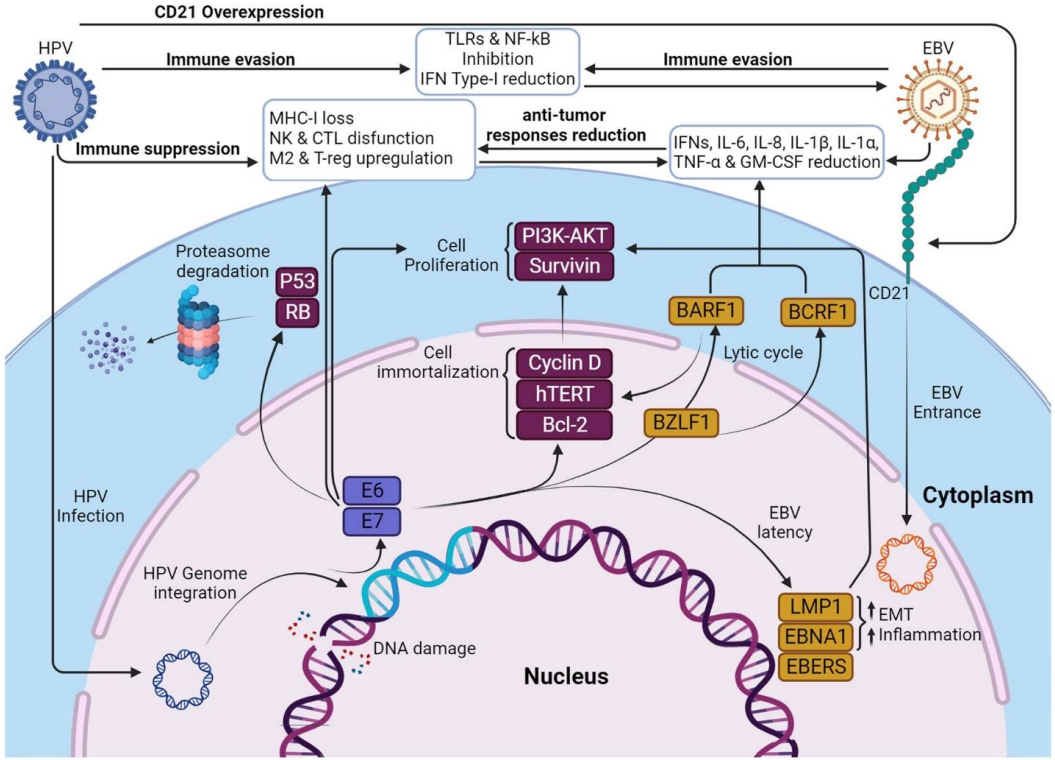
*Significant *p*-values

4.2 Potential carcinogenetic mechanism of EBV/HPV



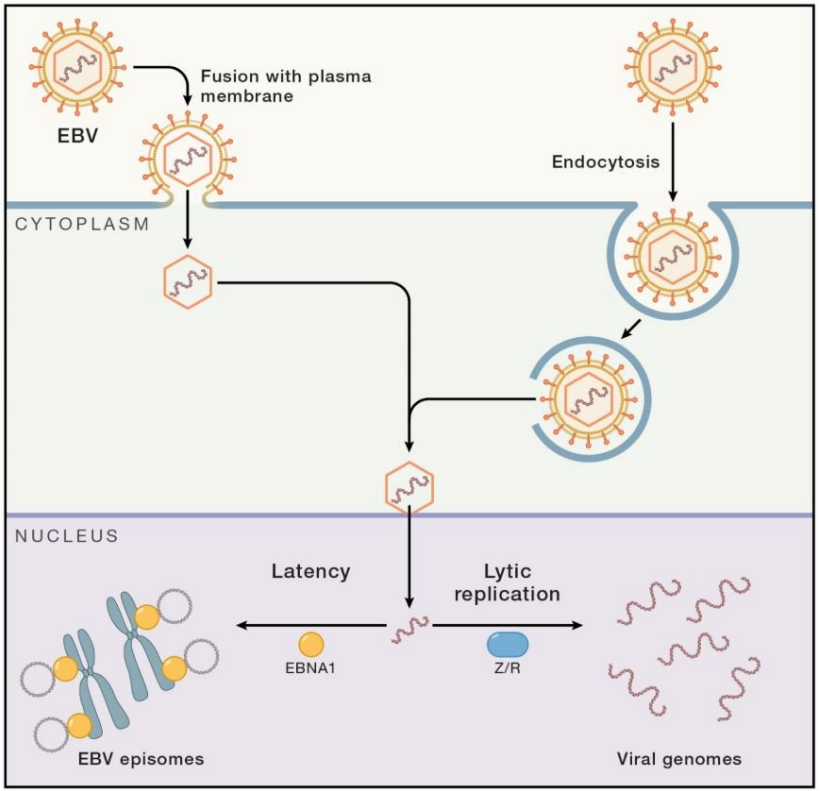
A model of carcinogenesis induced by high risk (HR)-HPV/EBV coinfection in the uterine cervix.

4.2 Potential carcinogenetic mechanism of EBV/HPV



A hypothetical model of HR-HPVs/EBV cooperation for the development of cancer.

4.2.1 HPV promotes EBV entry, latency and lytic cycle activation.

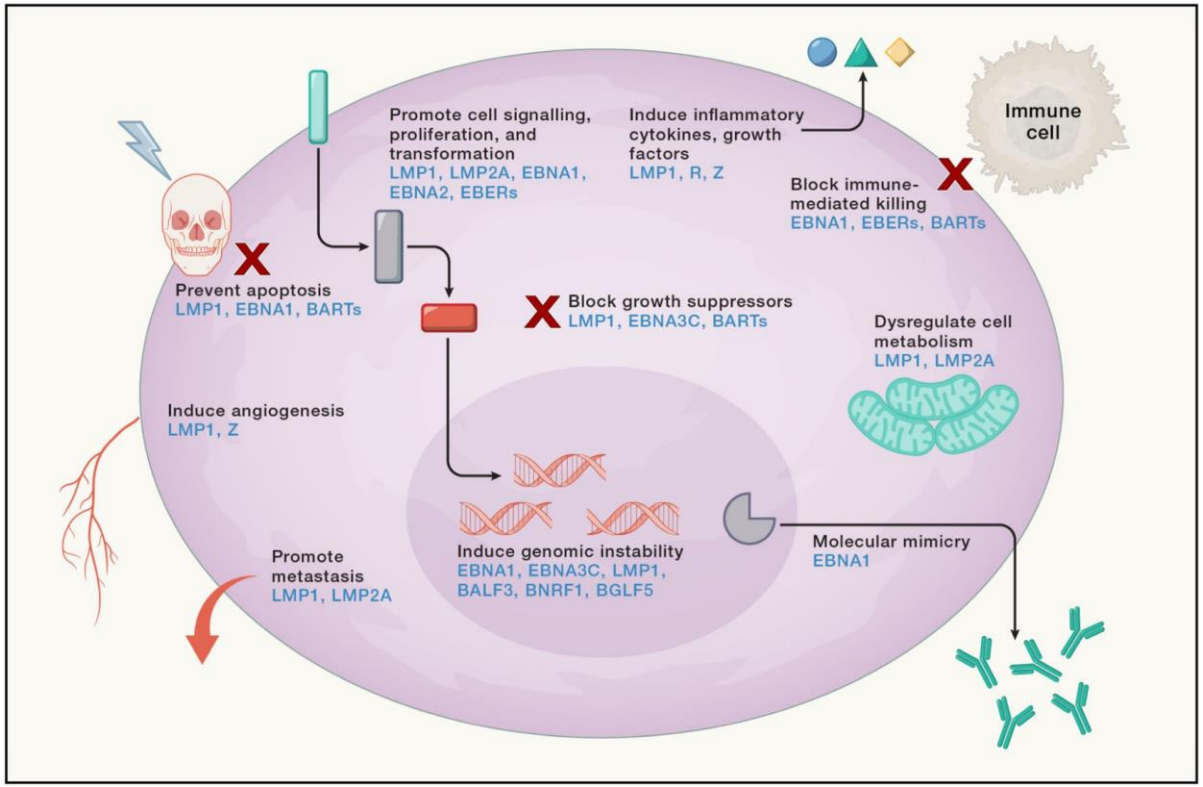


EBV viral entry.

- CD21 is responsible for EBV attachment and entry, CD21 expression level is higher in EBV/HPV+ cases compared to HPV/EBV- ones.
- In addition to CD21, the Ephrin receptor A2 -the epithelial EBV receptor, and C3d- another complement component binds to EBV in the cervix, is overexpressed in HPV-related cervical neoplasia (CN) compared to normal cervical tissue.
- HPV infection and its E6 and E7 oncogenes may play roles in the establishment of latent EBV infection and reduction of EBV replication by changing gene expression in EBV.

{Damania B, Cell, 2022. }

4.2.2 HPV/EBV mediates immune modulation, genome instability and cell proliferation.



EBV modulation of the infected cell.

{Damania B, Cell, 2022. }

5 Prevention and therapy

- Vaccines. For HPV: the 9-valent vaccine Gardasil 9® ; For EBV: a self-assembled nanoparticle vaccine contains 4 viral glycoproteins gp350/gH/gL/gp42 (in non-human primate pre-clinical study).
- For HPV/EBV coinfection: early screen of biomarkers, e.g. EBV latency proteins LMP1and LMP2, HPV E6 and E7 mRNA transcripts.
- For EBV therapy: *ex vivo* EBV-specific cytotoxic T-cells immune therapy.
- For HPV and HPV/EBV therapy: immune checkpoint inhibitors, e.g., PD-1 inhibitors.
- Monoclonal antibodies, targeting HPV E6 and E7 and EBV oncoproteins LMP1, LMP2, EBNA1, EBNA2.

{Wei CJ, Sci Transl Med. 2022.}

6 Summary

1. HR-HPV infection is associated with cervical cancer etc. EBV can cause lymphoma, carcinoma etc.
2. More evidences should be investigated into HPV/EBV co-infection as a contributing factor in cancers for the reason of limitations of cohort studies' sample size, region etc.
3. The synergistic carcinogenetic mechanism of EBV/HPV co-infection regards promotion of viral entry, immune suppression and evasion, genetic instability of viral oncogenes integration, activation of cellular proliferation and transformation pathways etc.
4. Latest prevention and therapy of EBV/HPV co-infection regards viral vaccines, early screen of biomarkers, immune therapy etc.

Reference

1. Hu M, Wang B, Li J, Wu C. Editorial: The association between viral infection and human cancers. Front Microbiol. 2024 Feb 2;15:1371581. doi: 10.3389/fmicb.2024.1371581. PMID: 38371929; PMCID: PMC10869602.
2. Bouvard, V., Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F., et al. (2009). A review of human carcinogens—part B: biological agents. Lancet Oncol 10, 321–322. doi: 10.1016/s1470-2045(09)70096-8.
3. Müller-Coan BG, Caetano BFR, Pagano JS, Elgui de Oliveira D. Cancer Progression Goes Viral: The Role of Oncoviruses in Aggressiveness of Malignancies. Trends Cancer. 2018 Jul;4(7):485-498. doi: 10.1016/j.trecan.2018.04.006. Epub 2018 May 21. PMID: 29937047.
4. Martinelli M, Musumeci R, Sechi I, et al. Prevalence of human papillomavirus (HPV) and other sexually transmitted infections (STIs) among Italian women referred for a colposcopy. Int J Environ Res Public Health. 2019;16(24):5000.
5. McBride AA. Human papillomaviruses: diversity, infection and host interactions. Nat Rev Microbiol. 2022 Feb;20(2):95-108.
6. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024 May-Jun;74(3):229-263. doi: 10.3322/caac.21834. Epub 2024 Apr 4. PMID: 38572751.zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology. 2009 Feb 20;384(2):260-5.
7. Scudellari M. HPV: Sex, cancer and a virus. Nature. 2013 Nov 21;503(7476):330-2.
8. Blanco R, et al. Human Papillomavirus in Breast Carcinogenesis: A Passenger, a Cofactor, or a Causal Agent? Biology (Basel). 2021 Aug 20;10(8):804.
9. Damania B, Kenney SC, Raab-Traub N. Epstein-Barr virus: Biology and clinical disease. Cell. 2022 Sep 29;185(20):3652-3670. doi: 10.1016/j.cell.2022.08.026. Epub 2022 Sep 15. PMID: 36113467; PMCID: PMC9529843.
10. Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, Chan SL, Chu SWI, Mak C, Tse IOL, Leung SYM, Chan G, Hui EP, Ma BBY, Chiu RWK, Leung SF, van Hasselt AC, Chan ATC, Lo YMD. Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med. 2017 Aug 10;377(6):513-522. doi: 10.1056/NEJMoa1701717. N Engl J Med. 2018 Mar 8;378(10):973. doi: 10.1056/NEJMx180004. PMID: 28792880.
11. Brodkin J, Kaprio T, Hagström J, Leppä A, Kokkola A, Haglund C, Böckelman C. Prognostic effect of immunohistochemically determined molecular subtypes in gastric cancer. BMC Cancer. 2024 Dec 2;24(1):1482Narisawa-Saito M, Kiyono T. Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: roles of E6 and E7 proteins. Cancer Sci. 2007 Oct;98(10):1505-11. doi: 10.1111/j.1349-7006.2007.00546.x. Epub 2007 Jul 23. PMID: 17645777; PMCID: PMC11158331.
12. Guidry JT, Scott RS. The interaction between human papillomavirus and other viruses. Virus Res. 2017;231:139-147.
13. Rahman R, Shaikh MH, Gopinath D, Idris A, Johnson NW. Human papillomavirus and Epstein-Barr virus co-infection in oral and oropharyngeal squamous cell carcinomas: a systematic review and meta-analysis. Mol Oral Microbiol. 2023;38(4):259-274.
14. Blanco R, Carrillo-Beltrán D, Corvalán AH, Aguayo F. High-risk human papillomavirus and Epstein–Barr virus coinfection: a potential role in head and neck carcinogenesis. Biology. 2021;10(12): 1232.
15. Guidry J, Birdwell C, Scott R. Epstein–Barr virus in the pathogenesis of oral cancers. Oral Dis. 2018;24(4):497-508.
16. Ghosh S, Shetty RS, Pattanshetty SM, et al. Human papilloma and other DNA virus infections of the cervix: a population based comparative study among tribal and general population in India. PLoS ONE. 2019;14(6):e0219173.
17. Sosse SA, Tadlaoui KA, Benhassou M, Elkarroumi M, Elmzibri M, Ennaji MM. Viral co-infection of oncogenic human papillomavirus with Epstein–Barr virus, human herpesvirus 8 and Herpes Simplex Virus type 2 in malignant cervical cancer. Int Med J. 2022;3(2):1-15.
18. de Lima MAP, Neto PJN, Lima LPM, et al. Association between Epstein-Barr virus (EBV) and cervical carcinoma: a meta-analysis. Gynecol Oncol. 2018;148(2):317-328.
19. Akbari E, Milani A, Seyedinkhorasani M, Bolhassani A. HPV co-infections with other pathogens in cancer development: A comprehensive review. J Med Virol. 2023 Nov;95(11):e29236. doi: 10.1002/jmv.29236. PMID: 37997472.
20. Jin YN, Yao JJ, Zhang F, Wang SY, Zhang WJ, Zhou GQ, Qi ZY, Sun Y. Is pretreatment Epstein-Barr virus DNA still associated with 6-year survival outcomes in locoregionally advanced nasopharyngeal carcinoma? J Cancer. 2017 Mar 12;8(6):976-982.
21. Li YY, Chung GT, Lui VW, To KF, Ma BB, Chow C, Woo JK, Yip KY, Seo J, Hui EP, Mak MK, Rusan M, Chau NG, Or YY, Law MH, Law PP, Liu ZW, Ngan HL, Hau PM, Verhoeft KR, Poon PH, Yoo SK, Shin JY, Lee SD, Lun SW, Jia L, Chan AW, Chan JY, Lai PB, Fung CY, Hung ST, Wang L, Chang AM, Chiosea SI, Hedberg ML, Tsao SW, van Hasselt AC, Chan AT, Grandis JR, Hammerman PS, Lo KW. Exome and genome sequencing of nasopharynx cancer identifies NF-κB pathway activating mutations. Nat Commun. 2017 Jan 18;8:14121.
22. Osorio JC, Blanco R, Corvalán AH, Muñoz JP, Calaf GM, Aguayo F. Epstein-Barr Virus Infection in Lung Cancer: Insights and Perspectives. Pathogens. 2022 Jan 21;11(2):132. doi: 10.3390/pathogens11020132. PMID: 35215076; PMCID: PMC8878590.
23. Hariwiyanto B, Sastrowiyoto S, Mubarika S, Salugu M. LMP1 and LMP2 may be prognostic factors for outcome of therapy in nasopharyngeal cancers in Indonesia. Asian Pac J Cancer Prev. 2010;11(3):763-6. PMID: 21039050.
24. Fernandes Q, Gupta I, Murshed K, Samra HA, Al-Thawadi H, Vranic S, Petkar M, Babu GR, Moustafa AA. Incidence and association of high-risk HPVs and EBV in patients with advanced stages of colorectal cancer from Qatar. Hum Vaccin Immunother. 2023 Aug 1;19(2):2220626. Fatemeh Ebrahimi, et al. Coinfection of EBV with other pathogens: a narrative review. Frontiers in Virology. 2024
25. Tung YC, Lin KH, Chu PY, Hsu CC, Kuo WR. Detection of human papilloma virus and Epstein-Barr virus DNA in nasopharyngeal carcinoma by polymerase chain reaction. Kaohsiung J Med Sci. 1999 May;15(5):256-62. PMID: 10375867.
26. Al-Thawadi H, Gupta I, Jabeen A, Skenderi F, Aboulkassim T, Yasmeen A, Malki MI, Batist G, Vranic S, Al Moustafa AE. Co-presence of human papillomaviruses and Epstein-Barr virus is linked with advanced tumor stage: a tissue microarray study in head and neck cancer patients. Cancer Cell Int. 2020 Aug 3;20:361. doi: 10.1186/s12935-020-01348-y. PMID: 32774155; PMCID: PMC7397600.
27. Wei CJ, Bu W, Nguyen LA, Batchelor JD, Kim J, Pittaluga S, Fuller JR, Nguyen H, Chou TH, Cohen JI, Nabel GJ. A bivalent Epstein-Barr virus vaccine induces neutralizing antibodies that block infection and confer immunity in humanized mice. Sci Transl Med. 2022 May.

香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Thank you

Supplement 1: HPV proteins

Table 1 | **Key functions of human papillomavirus proteins**

Protein	Function and characteristics	Role in infection
E1	Origin-binding DNA helicase[117] Site-specific DNA-binding protein[118,119]	Initiates viral DNA replication and recruits cellular replication machinery[120,121]
E2	Dimeric DNA-binding protein that binds E2-binding sites in viral DNA[119] Interacts with and loads the E1 DNA helicase onto the replication origin[122] Tethers viral DNA to the host chromatin[123]	Regulates viral transcription[124] Supports viral DNA replication[121] Partitions viral genomes to daughter cells[125]
E8^E2	Site-specific DNA-binding protein that binds E2-binding sites in viral DNA[56] Interacts with cellular transcriptional repressor factors[56]	Represses viral transcription and replication to maintain low-level persistent infection[56]
E1^E4	Highly expressed late protein[59] Induces G2 cell cycle arrest[126] Disrupts and reorganizes keratin filaments[58]	Promotes viral genome amplification[59] Causes fragility in keratinocyte squames to promote viral egress[58]
E5	Hydrophobic membrane proteins[60] Four types[53] Encoded only by Alphapapillomaviruses	Reduces immune detection[61] Promotes cell proliferation and productive stages of infection[60]

Table1A. Key functions of HPV proteins.
{McBride, Nat Rev Microbiol, 2022. }

Supplement 1: HPV proteins

E7	Binds and inactivates Rb protein (pRb) and related pocket proteins to promote cell cycle entry[62] Degrades pRb (high-risk HPVs only)[62] Induces DNA damage response signalling in differentiated cells[127] Abrogates the innate immune response[55]	Promotes cell proliferation and viral DNA amplification in differentiating cells[55] Decouples response to oncogene-induced senescence[55] Promotes genome amplification[18] Reduces immune detection[55]
E6	In Alphapapillomaviruses: Inhibits p53 function[55,108] Binds E6-associated protein E3 ubiquitin ligase[128] Degrades p53 (high-risk HPVs only)[55,129] Degrades PDZ domain-containing proteins involved in cell polarity (high-risk HPVs only)[108] Activates telomerase (high-risk HPVs only)[64]	Prevents growth arrest and apoptosis[55] Decouples response to oncogene-induced senescence[55] Modulates cell polarity to promote viral genome replication and maintenance[55]
	In Betapapillomaviruses: Inhibits p53 function[110] Inhibits DNA damage response[110] Binds MAML1 to inhibit NOTCH signalling[63,65]	Prevents replicative senescence[64] Abrogates immune signalling[55] Prevents growth arrest and apoptosis in response to UV irradiation[66] Inhibits keratinocyte differentiation[63,65] Abrogates immune signalling[55]
L1	Capsid protein that self-assembles into capsids consisting of 360 L1 proteins[69]	Major capsid protein[69]
L2	Between 12 and 72 L2 proteins per capsid[130] Traffics viral genome into nucleus, associates with host chromosomes and PML nuclear bodies in early infection[80] Packages viral genome into capsids at late stages of infection[105]	Minor capsid protein[131] Viral genome chaperone[67]

Table1B. Key functions of HPV proteins.
{McBride, Nat Rev Microbiol, 2022. }

Supplement 2: HPV HR/LR

Group	HPV types	Comments
Alpha HPV types		
1	16	Most potent HPV type, known to cause cancer at several sites
1	18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	Sufficient evidence for cervical cancer
2A	68	Limited evidence in humans and strong mechanistic evidence for cervical cancer
2B	26, 53, 66, 67, 70, 73, 82	Limited evidence in humans for cervical cancer
2B	30, 34, 69, 85, 97	Classified by phylogenetic analogy to HPV types with sufficient or limited evidence in humans
3	6, 11	..
Beta HPV types		
2B	5 and 8	Limited evidence for skin cancer in patients with epidermodysplasia verruciformis
3	Other beta and gamma types	..

Table 2: **Human papillomavirus (HPV) types assessed by the IARC Monograph Working Group**