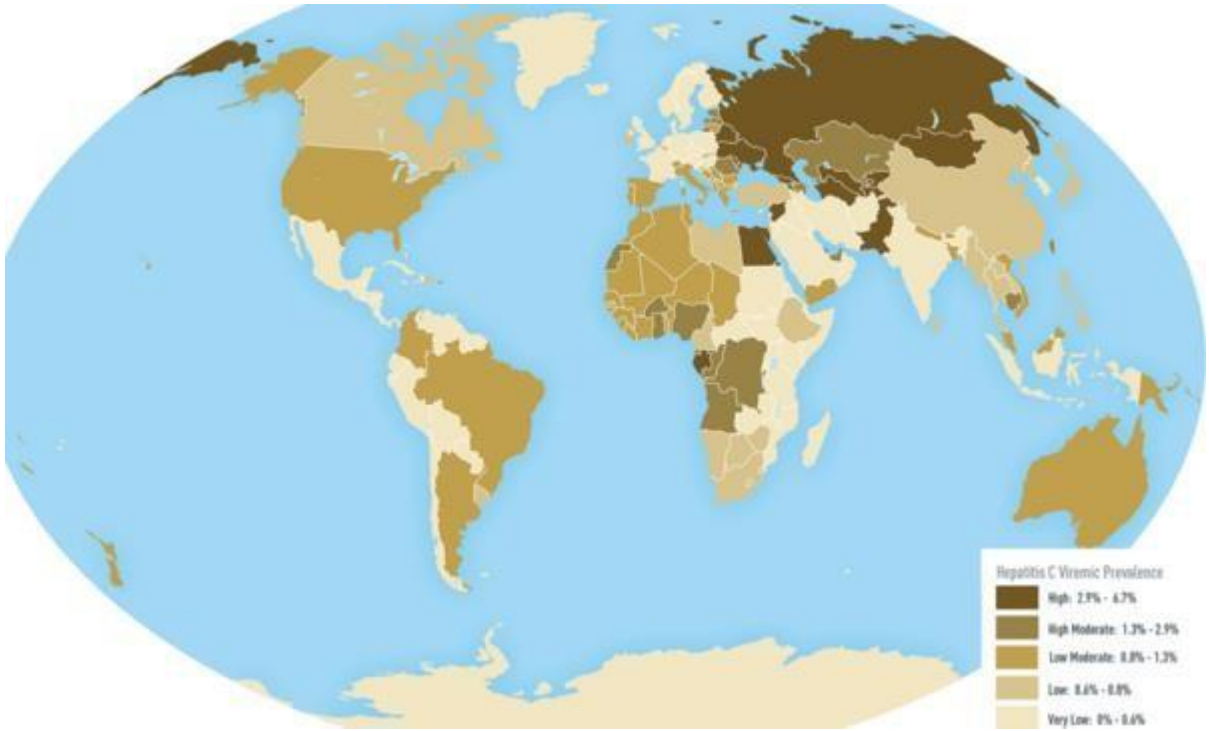
ONCOGENIC VIRUSES

Teaching unit 08

Oncogenic viruses

- Viral infections play a role in development at least 10% of all newly diagnosed tumors in the world.
- Most cases of tumors whose origin is associated with previous viral infections (> 85%) occurs in developing countries, where poor sanitary conditions, high rates of co-carcinogenic factors, lack of vaccines and lack of screening contribute to the increase in the rate of these tumors.
- Even in developed countries, where effective counter measures are widely available, tumors whose development is influenced by a previous viral infection account for at least 4% of new cases.



Prevalence of HCV infection

Oncogenic viruses that infect humans come from six different virus families but with a number of similar physical characteristics

Oncogenic viruses						
A virus	Family	Genome	Virion	Rate infections	A place of persistence	Tumors
HPV 16 and 18	Papillomaviridae	circular double stranded DNA	Non-enveloped	> 70%	ano-genital and oral mucosa	Cancer of cervix, anus vulva, penis, vagina, tongue, urinary bladder
HBV	Hepadnaviridae	incomplete double stranded DNA	enveloped	2-8%	hepatocytes	Hepatocellular carcinoma
HCV	Flaviviridae	+ RNA	enveloped	<3%	hepatocytes	Hepatocellular cancer, lymphoma marginal zones of the spleen
Epstein-Barr virus (HHV-4)	Herpetoviridae	linear double stranded DNA	enveloped	90%	Blymphocytes, pharyngeal mucosa	Burkitt's lymphoma, second non-Hodgkin's lymphomas, nasopharyngeal carcinoma
Kaposi sarcoma herpesvirus (HHV-8)	Herpetoviridae	linear double stranded DNA	enveloped	2-60%	Oral mucosa, endothelium, B lymphocytes	Kaposi sarcoma, multicentric Castleman's disease
Merkel cell polyomavirus	Polyomaviridae	circular double stranded DNA	Non-enveloped	75%	skin	Merkel's cell cancer
HTLV	Retroviridae	+ RNA	enveloped	0.01-6%	T and V lymphocytes	Leukemia and lymphoma of T lymphocytes

Oncogenic viruses

- All known oncogenic viruses that infect humans are capable to establish permanent, long-term infections but cause tumors only in a small number of permanently infected persons.
- Slow tumor development (usually for many years after the initial infection) suggests that viral infection alone is rarely sufficient to cause malignancy and that a tumor whose development is associated with viral infection arises only after additional oncogenic stimuli have had time to accumulate.
- The first findings that indicated that HPV, EBV, KSHV, and MCV may be associated with tumor development are based on the detection of virions, viral DNA, or viral RNA in tumors.

Oncogenic viruses

- A common characteristic of all known tumors whose origin is related to a viral infection is their higher prevalence in the population of immunodeficient persons, and it is a consequence of inadequate control of the viral infection.

•The discovery that a virus can be one of the causes of the appearance of tumors can suggests

- possible ways of clinical intervention (development of vaccines or antiviral agents that prevent, reduce or eradicate viral infection and thus prevent the development of tumors);
- development of methods for early detection or diagnosis of tumors based on testing of viral nucleic acids or viral gene products; or
- development of drugs or immunotherapeutic interventions for the treatment of tumors by targeting viral gene products.

Oncogenic viruses

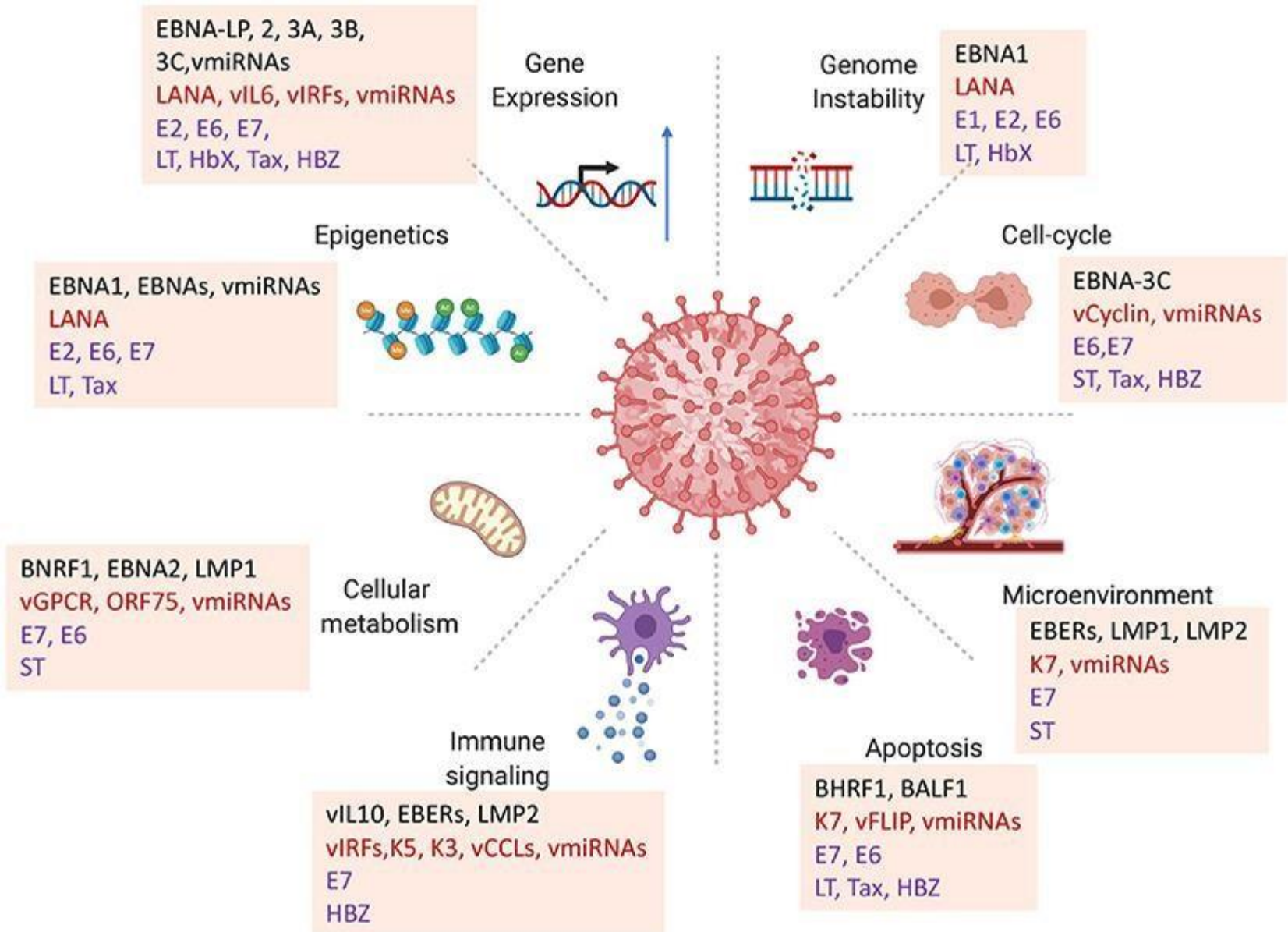
- Viruses can affect the development of tumors on one (or both) of two known mechanisms:

  - Direct (implying the expression of the viral oncogene in the cell or direct genotoxic effect of viral proteins)
  - Indirect (cells that undergo malignant transformation are never infected with a virus, but a viral infection by inducing an inflammatory response promotes accelerated tissue damage and subsequent regeneration)

Direct mechanisms of cell transformation by viruses

- A common characteristic of DNA viruses whose replication depends on the DNA polymerase of the host´s cells (eg papilloma- and polyoma-viruses) is the expression of viral genes whose products promote cell progression through the cell cycle.
- A typical mechanism of direct oncogenic effects of viruses is the inactivation of tumor suppressor proteins, p53 and pRb, so the cell division is stimulated and cell begins to produce enzymes necessary for viral DNA replication. The study of oncogenic viruses has been instrumental in revealing the existence and function of key tumor suppressor proteins, as well as key cellular proto-oncogenes, such as Src and Myc.
- Hit-and-run mechanism: viral gene products can promote growth and survival cells that are, during the early stages of tumor development, faced with gene damage that acts proapoptotic, so precancerous cells can accumulate enough additional gene mutations that cell growth and survival is possible independently of viral oncogene expression. Thus, a stochastic loss of viral nucleic acids from the newly formed tumor is possible, which may enable the faster growth of those cells, because the loss of "foreign" viral antigens also leads to the loss of stimulation of the host's immune response, which normally has a role in removing tumor-altered cells.
- This hit-and-run mechanism has been reported in bovine gastrointestinal carcinoma caused by bovine papillomavirus and has been proposed for the development of human cancers associated with hepatitis B and Cviruses, *Epstein-Barr* virus and human papilloma viruses, but it is not experimentally confirmed.

Numerous possibilities of the oncogenic effect of the virus



Determining the oncogenic potential of viruses

- Demonstration that a virus can transform cells in culture and/or cause a tumor in animals provides indirect evidence of the virus's oncogenic potential (all known human oncogenic viruses, except KSHV, meet this criterion)

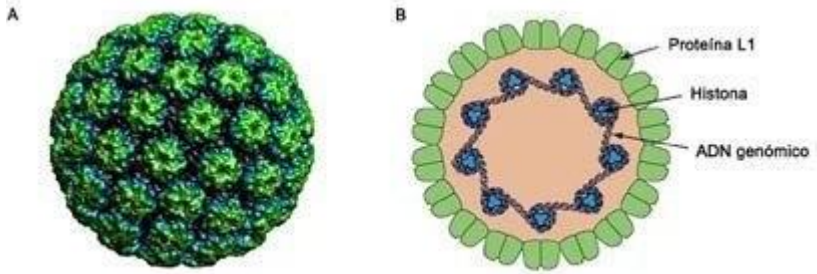
- however, it is important to recognize that viruses can theoretically be non-oncogenic for their natural host (e.g. humans) and cause tumors only in the host organism that they do not infect under natural conditions (e.g. animals), which is the case with human adenoviruses

- The finding that viral DNA is clonally integrated into the primary tumor and its metastatic lesions can indicate that the virus may just be using the tumor cell as a convenient environment for replication (without having a causal role). However, in most cases, it has been shown that viruses found in tumors have lost the ability to exit latency and are functionally unable to replicate. Hence, vaccines or antiviral drugs that target virion proteins (e.g. vaccines against high-risk HPV or HBV) or products of viral genes that are expressed late in the viral life cycle (e.g. herpesvirus thymidine kinase that targets drugs such as ganciclovir) are rarely effective in treating existing tumors whose development is influenced by viruses.

Determining the oncogenic potential of viruses

- Showing that a vaccine or antiviral drug that targets the virus, prevents or cures a tumor, is the strongest form of evidence that a given virus causes a tumor in humans.
- This type of evidence fully supports a causal role of HBV in the development of liver tumors in humans.
- Clinical trial data also show that antiherpesviral therapeutics can prevent the development of lymphoproliferative diseases associated with KSHV or EBV infection and that HPV vaccination can prevent the development of precancerous cervical lesions.

Papillomaviridae



•In the 1930s, Richard Schope demonstrated the transmission of papillomavirus in rabbits.

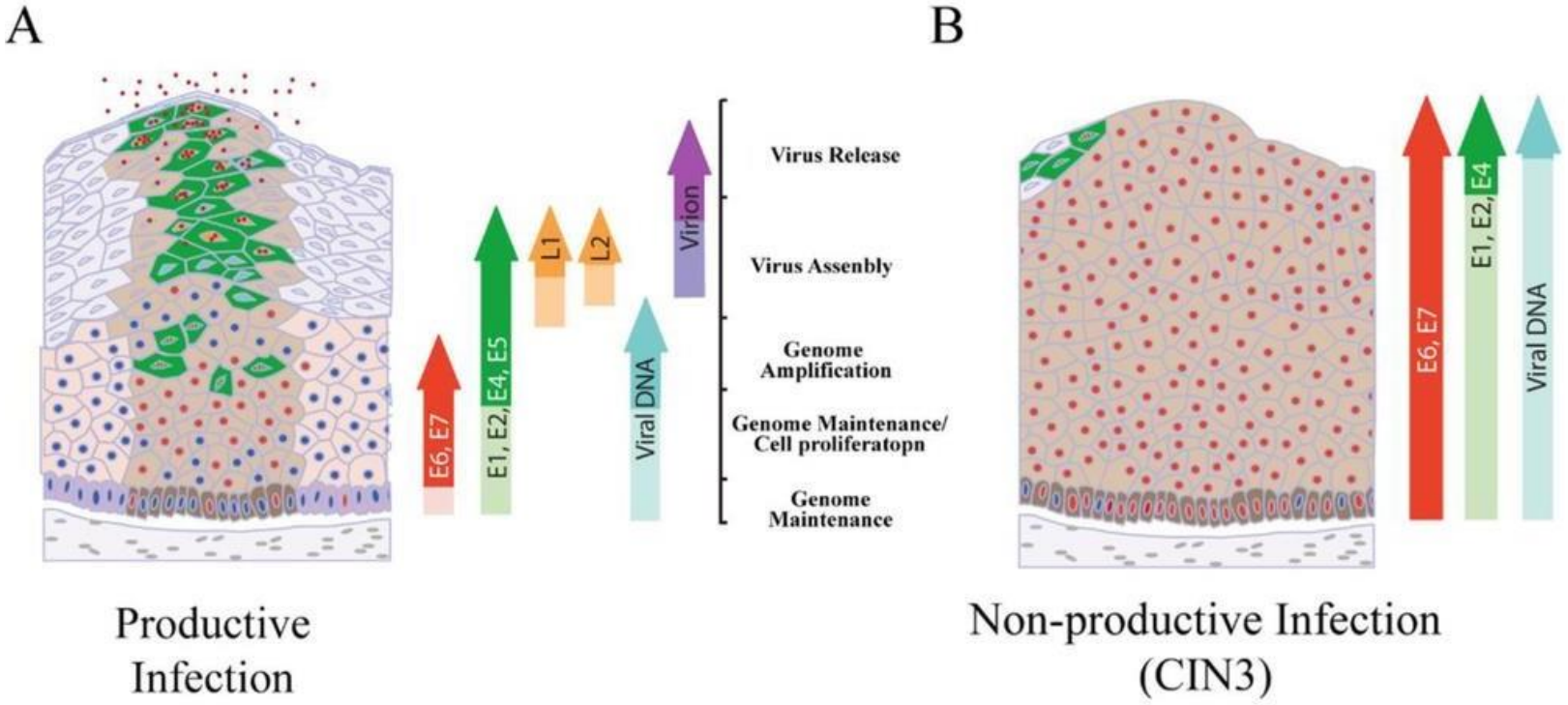
- Using this system, Peyton Rouse showed that lesions induced in this way on rabbit tail can progress to a malignant skin tumor.
- Dominico Rigoni-Stern proposed in the mid-19th century that cervical cancer may be related to sexual behavior.
- Harald Hausen discovered the presence of two previously unknown papilloma viruses, HPV16 and HPV18, in various cervical cancer cell lines, including the well-known HeLa cell line. Hausen won the Nobel Prize in 2008 for his work establishing the link between HPV and human cancer.

Papillomavirus tropism

- Although papillomaviruses can infect a large number of different cells *in vivo and in vitro the* late stage of the viral cycle, during which the viral DNA is replicated and the genes encoding the structural proteins L1 and L2 are expressed, strictly depends on factors from host cells that are found only in differentiated keratinocytes of the skin or mucosa.

- Most cancers that are associated with HPV infection occurs primarily in the transition zones between the squamous-layered and single-layered cylindrical epithelium of the endocervix, the inner surface of the anus and the crypt of the tonsils, the mixed phenotype of cells in the squamocolumnar transition zones can cause a disruption of the regulation of the normal life cycle of HPV, which depends on the differentiation of keratinocytes.

HPV infections may or may not be associated with the formation of visible warts or other lesions.

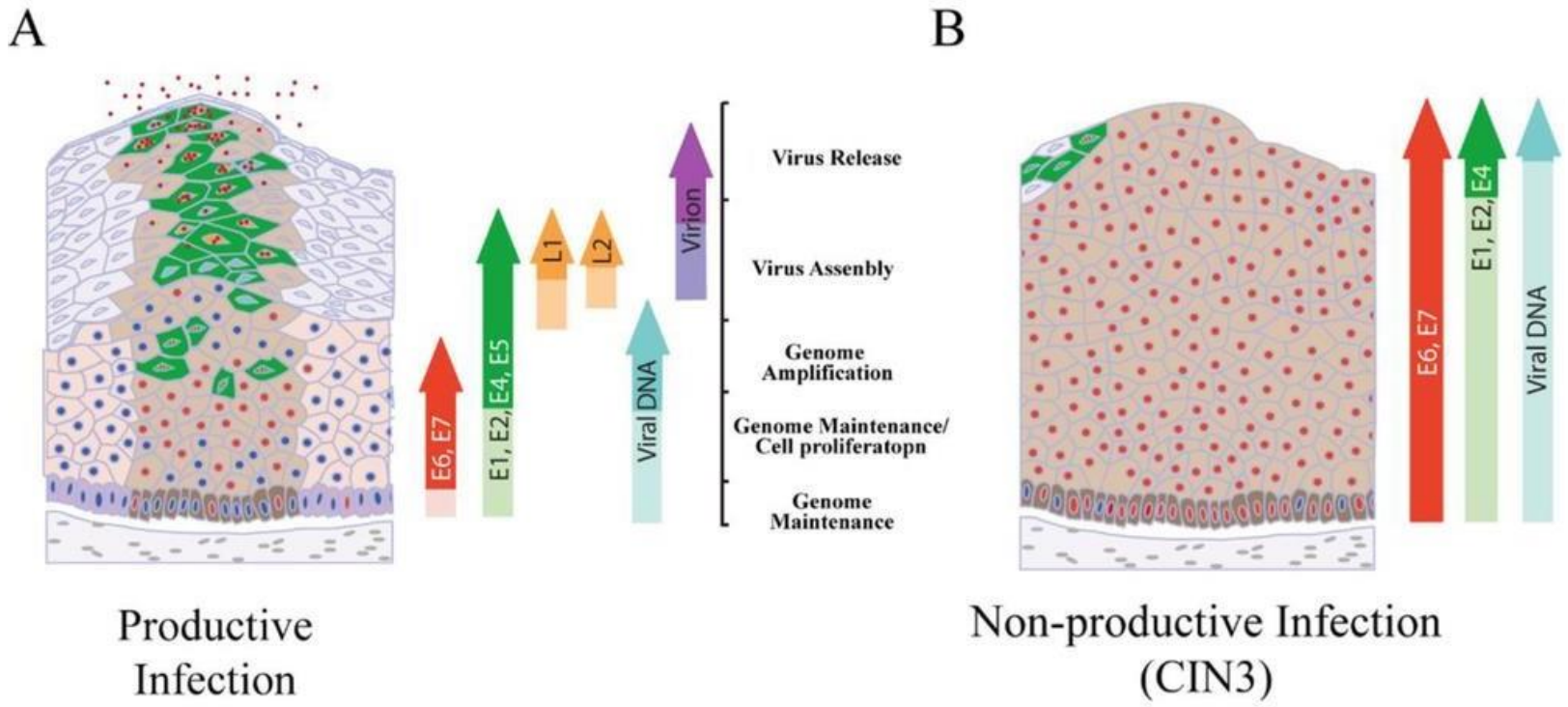


Papillomavirus tropism

High-risk types of HPV, with clearly established causal links with the development of tumors in humans, show a tropism primarily for the anogenital and oral mucosa, are usually transmitted by sexual contact, rarely cause visible warts, and usually establish only transient infections in the vast majority of cases.

It is estimated that an individual's lifetime risk of sexual exposure to a high-risk type of HPV is > 70%.

Individuals who fail to eliminate high-risk HPV infection and remain infected have a much higher risk of developing cancer. Screening based on the detection of the presence of high-risk types of HPV by the PCR method serves as an addition or replacement for the traditional Pap test.

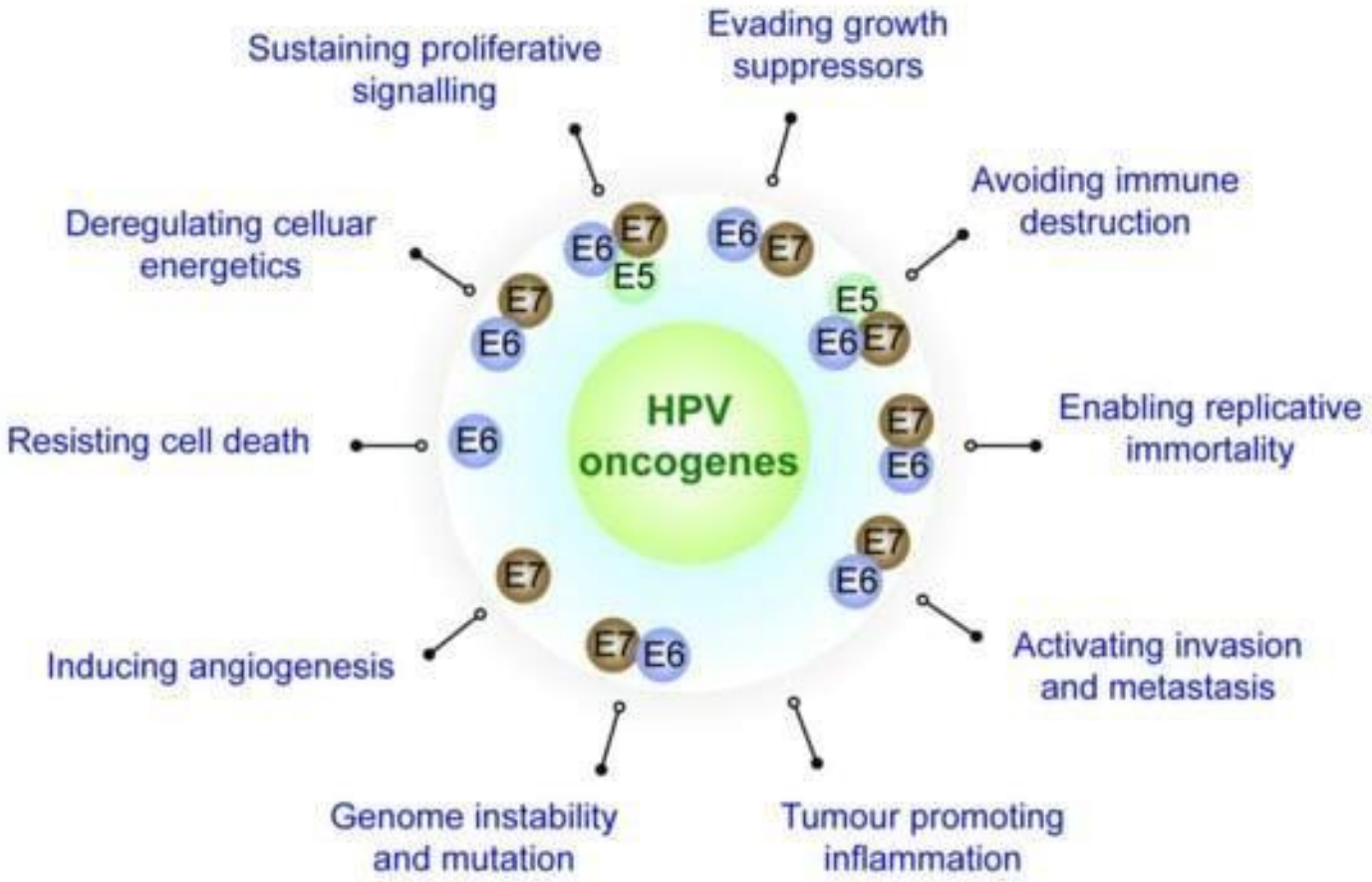


Functions of viral gene products

The most extensively studied early proteins are the oncogenes E6 and E7 of the oncogenic variants HPV 16 and HPV 18.

•E6 high-risk HPV protein for tumor development:

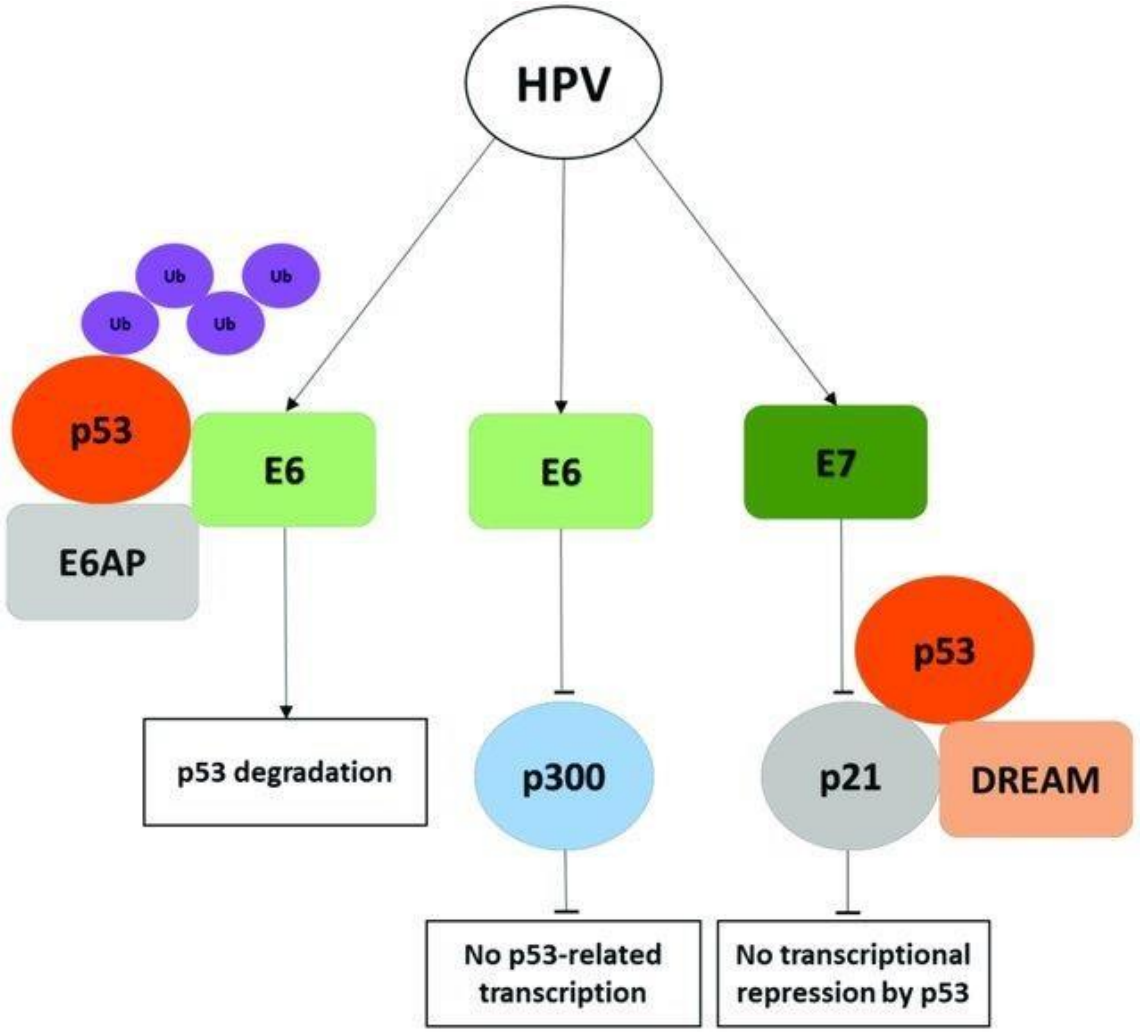
- initiates the elimination of p53 by recruiting the host cell ubiquitin ligase, E6AP
- activates cellular telomerase.



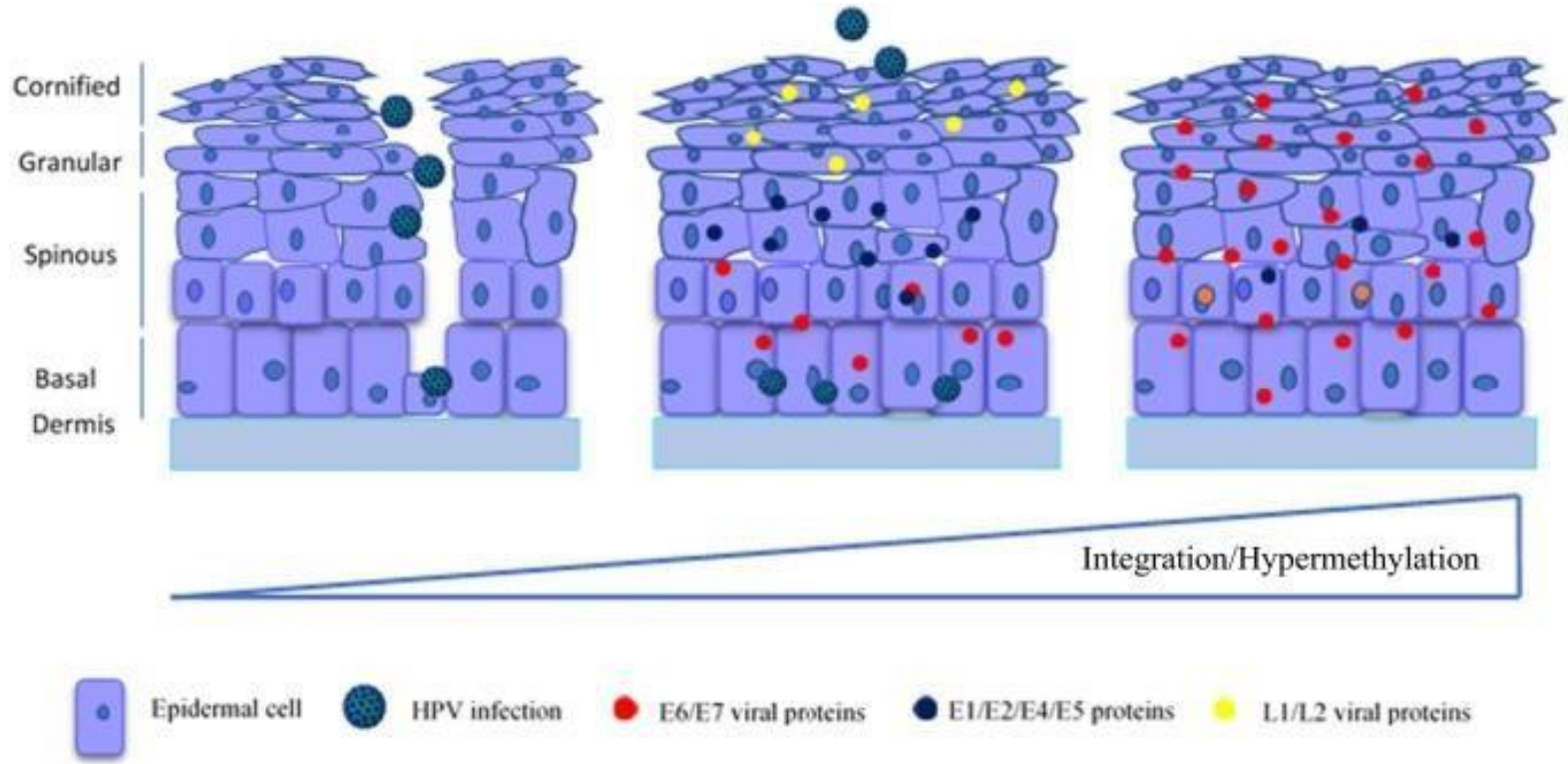
Functions of viral gene products

- Most proteins of E7, including those of many low-risk HPV types:
  - contain preserved the LXCXE motif that mediates an interaction with pRb that disrupts complex formation between the transcription factor pRB and E2F, thereby blocking the ability of pRB to arrest the cell cycle.
  - E7 proteins of high-risk HPVs may also contribute to chromosome missegregation and aneuploidy, which may contribute to malignant transformation and progression.

- Some types of papillomavirus express oncoprotein E5, which acts as an agonist for growth factor receptors such as platelet-derived growth factor β (PDGF-β) and epidermal growth factor (EGF), E5 expression is uncommon in cervical tumors and it remains uncertain whether the protein plays a key role in tumor development in humans.



HPV carcinogenesis



Human papillomavirus vaccines

- Two preventive vaccines for HPV with oncogenic potential are available

- These vaccines contain recombinant L1 capsid proteins from HPV 16 and 18 variants that assemble into virus-like particles *in vitro*, are highly immunogenic and cause a strong humoral immune response, which is manifested by a large production of specific antibodies capable of neutralizing the infectivity of related HPV types.

- Like keratinocyte stem cells, cervical cancer cells and precursor lesions rarely or never express L1, so it appears that existing L1-based vaccines cannot serve as therapeutic agent for the treatment of cervical cancer.

- Another category of vaccines induces a T lymphocyte immune response to oncoproteins E6 and E7. Since the expression of E6 and E7 is crucial for tumor development and survival, such vaccines can also be used therapeutically to eradicate precancerous lesions and treat cervical cancer.

Carcinoma of the oropharynx

- It has been shown that tobacco smoke and alcohol can be associated with development of head and neck tumors, and at the end of the nineties of the twentieth century, a surprising number of new cases of tonsil tumors were observed in non-smokers.
- Many tonsil tumors of non-smokers have been found to have the wild-type of p53 allele, which indicates that the tumor may be dependent on a viral oncogene that suppresses the function of p53 (as shown in cervical cancer).
- Almost half of all tonsil cancers have been shown to contain DNA of HPV, most often HPV16.

Interestingly, HPV-positive oropharyngeal carcinomas usually have a lower mortality rate compared to HPV-negative tumors associated with tobacco use, which has important implications for the treatment of HPV-positive head and neck cancer.

Non-melanoma skin cancer

- Epidermodysplasia veruciformis is a rare immunodeficiency, characterized by the appearance of numerous wart-like lesions that cover large areas of the skin.
- Lesions usually contain papillomaviruses such as HPV5 or HPV8.
- Patients with epidermodysplasia veruciformis often develop squamous cell carcinoma in areas of sun-exposed skin (suggesting that exposure to ultraviolet light is a cofactor).
- It is also well established that other immunodeficient persons, such as transplant recipients and people infected with HIV, are at an increased risk of developing squamous cell carcinoma.
- Sequencing studies have shown that these tumors have little or no viral sequences, indicating no permanent direct oncogenic effects of any known viral species but suggesting that papillomaviruses may be associated with tumor development by a hit-and-run mechanism.

Polyomaviruses

- At the beginning of the 1950s, Ludwik Gross showed that an infectious agent could cause salivary gland cancer in laboratory mice.
- Later work by Bernice Eddy and Sarah Stewart showed that murine polyoma (Greek for "many tumors") virus causes many different types of tumors in experimentally infected mice.
- These viruses were initially, due to their similarity to papillomaviruses, placed in a common family, the Papovaviridae, but sequencing studies eventually revealed that polyomaviruses have a unique genome organization (with early and late genes arranged on opposite strands of the genome) and essentially no nucleotide sequence similarity to the papillomaviruses, two groups of viruses are thus divided in separate families.

SV40

- At the beginning of the 1960s, Bernice Eddy, Maurice Hilleman, and Benjamin Sweet reported the discovery of simian vacuolating virus 40 (SV40), which was found to be contaminating poliovirus vaccines.
- SV40 was derived from rhesus monkey kidney cells that were used to replicate poliovirus in culture.
- Studies of major and minor tumor antigens of SV40s have played an important role in understanding various aspects of carcinogenesis.
- But no studies have been able to find convincing evidence that exposure to SV40 is causally linked to human cancer.

BKV and JCV

- BKV and JCV cause kidney disease and a fatal brain disease, progressive multifocal leukoencephalopathy in immunosuppressed individuals.
- Like SV40, BKV and JCcan cause various forms of cancer in experimentally exposed animals.
- Integrated sequences of BKV has been documented in two tumors from a panel of 412 invasive bladder cancers, found in many cases of urinary tract carcinoma developed in transplant recipients, several recent epidemiologic studies have shown that post-kidney transplant patients with clinically documented failure to control BKV replication have significantly increased risk of developing invasive bladder cancer, which together strongly suggests that BKV plays a direct carcinogenic role in a small percentage of bladder cancers.
- There is a possibility that BKV plays a hit-and-run role in a certain percentage of urinary epithelial cancers.

Merkel cell polyomavirus

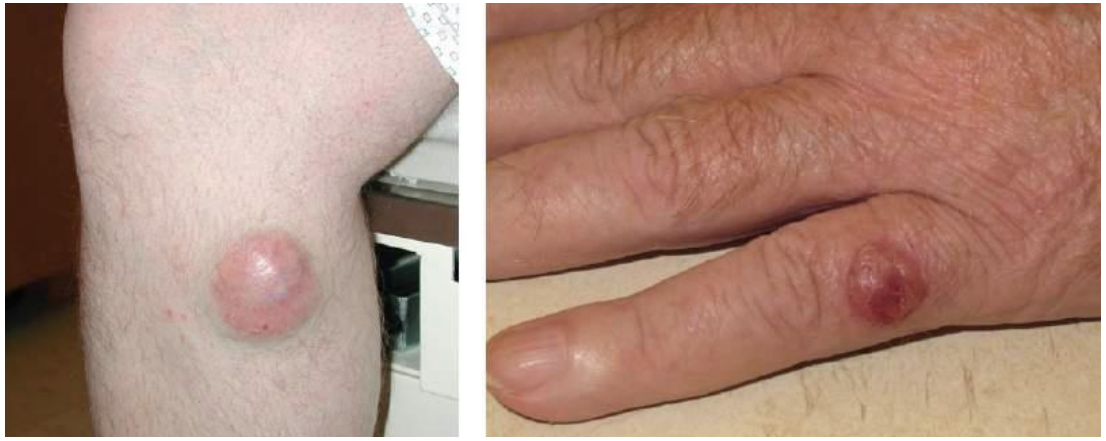
- In 2008, Yuan Chang and Patrick Moore reported the discovery of the fifth known human type of polyomavirus, which they named MCV based on its presence in Merkel cell carcinoma (MCC).

- MCC is a rare but deadly form of cancer that usually presents as a fast-growing purple lesion on sun-exposed skin surfaces.

- Risk of MCC is dramatically higher in AIDS patients and in people after organ transplants, which are initial indications that MCC might be a tumor caused by a virus. In 2012, the International Agency for Research on Cancer classified MCV as a class 2A oncogenic virus (probably carcinogenic to humans).
- The vast majority of healthy adults have serum antibodies specific for the major capsid protein of MCV, but patients with MCC have extremely high titers of these antibodies.

Merkel cell polyomavirus

- The LT protein of polyomavirus contains the LXCXE motif that participates in the inactivation of pRb function. LT protein does not deactivate p53 function, but activates responses to damaged DNA and induces cell cycle arrest in cell culture.
- People from MCCs that have more copies of MCV DNA in tumors, higher expression of tumor antigens in tumors that are infiltrated with CD8+ T cells have better prognosis.
- This is consistent with the idea that immuneCell-mediated immunity may help eliminate MCC tumors expressing MCV antigens.

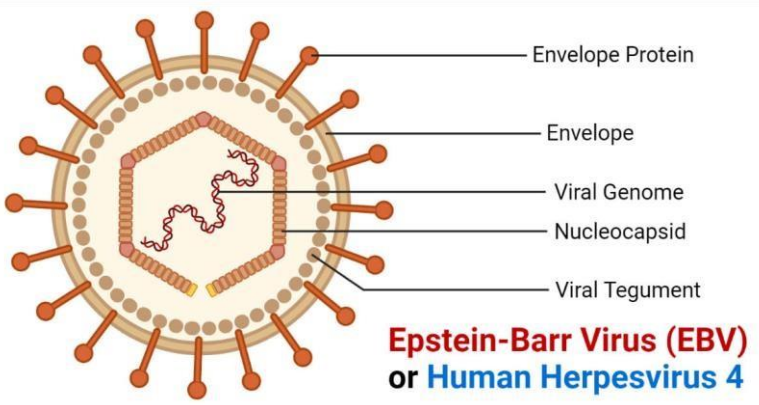


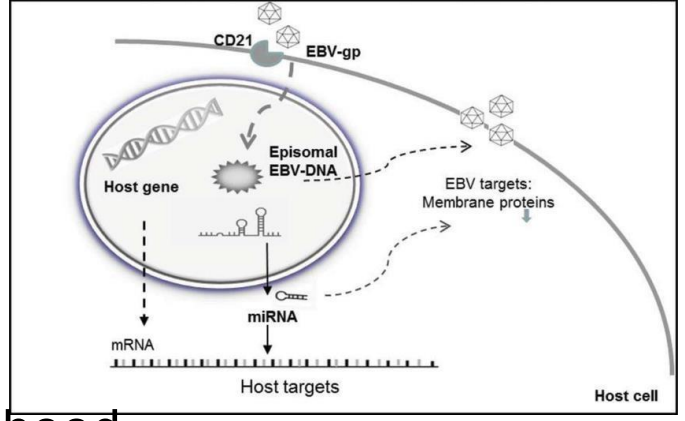
Merkel cell carcinoma

Epstein-Barr virus, the discovery of the association with the development of tumors

- In 1958 Dennis Burkitt described an unusual tumor of B lymphocyte that most often occurs in the jawbones of children in equatorial Africa.
- The first description of this tumor dates in 1896, when Albert Cook, a medical missionary in Uganda, reported a child with a large jaw mass.
- Epstein and Barr showed viral particles with a morphology strikingly similar to herpes simplex viruses in electron micrographs of tumors cells grown in culture. Soon it turned out that *Epstein-Barr virus* (EBV, later designated as human herpesvirus 4) can immortalize primary human B lymphocytes *in vitro*, creating constantly proliferating lymphoblastoid cells.
- Although more than 90% of adults in the world is asymptomatically infected with EBV and less than 50% of cases of sporadic forms of Burkitt's lymphoma are positive for EBV, there is a consensus that EBV is an oncogenic virus.
- The World Health Organization declared EBV is a first-class carcinogen.

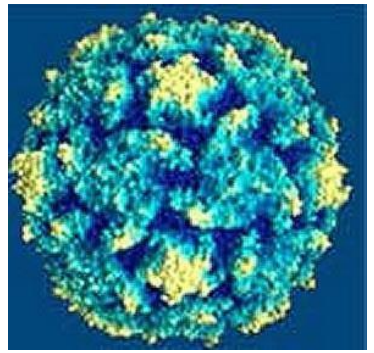
EBV infection





- In most individuals, EBV infection occurs asymptomatically in early childhood.
- Virus envelope glycoprotein gp350 binds with high affinity to the complement receptor, CD21, expressed by B lymphocytes. This interaction is accompanied by the entry of the virus into VB lymphocytes and the establishment of a long-term non-productive infection.
- Individuals who avoid infection with this virus during childhood, and meet the virus during adolescence or adulthood, often develop infectious mononucleosis, and a high viral load and are at increased risk of developing EBV-positive Hodgkin's lymphoma.
- After primary infection, EBV persists in the host by establishing latency in a small number of resting B lymphocytes and periodically reactivates, mainly in the oropharyngeal epithelium.
- Primary and chronic EBV infection is controlled by the host's innate and acquired immune response. EBV, like other herpesviruses, expresses a number of proteins that interfere with the host's T lymphocyte-mediated immune response which, together with its ability to establish latency, could explain why the virus cannot be eradicated after primary infection.

Epstein-Barr virus



- EBV is a polyclonal activator of V lymphocytes and causes their immortalization.
- Overexpression of EBNA-1 occurs in Burkitt's lymphoma, in Non-Burkitt lymphoma cells overexpression of EBNA-2, EBNA-5, LMP-1 and LMP-2, and in nasopharyngeal carcinoma cells EBNA-1, LMP-1 and LMP-2.
- EBNA-1 is associated with latent infection and persistence of EBV genome in episomal form.
- EBNA-2 is a transactivator of viral and cellular promoters and together with EBNA-5 protein activates G1 cyclin which is an essential event in B cell immortalization.
- EBNA-5 inactivates p53 and Rb protein.
- LMP-1 and LMP-2 are transactivators of cellular genes, by transactivating the bcl-2 gene inhibit programmed cell death.

Lymphomas and EBV

- Almost all cases of endemic Burkitt's lymphoma are EBV-positive.
- On the contrary, EBV is present in only about 20% of sporadic cases of Burkitt's lymphoma that occur in immunocompetent individuals outside regions where malaria is endemic.

- It is known that persons infected with HIV have a 60 to 200 times greater risk of developing Burkitt's and other non-Hodgkin's lymphomas, and about half of the lymphomas in people with HIV contain EBV.

- A hallmark of all types of Burkitt's lymphoma is the deregulation of the cellular proto-oncogene Myc. The classic mutation involves a chromosomal translocation of the Myc gene to the antibody heavy chain locus.

  t(8;14)(q24;q32)

- Burkitt's lymphomas lacking EBV DNA tend to have more additional mutations. This is consistent with a tumorigenic role for EBV and suggests the possibility of a hit-and-run scenario in which an originally EBV-positive cell has accumulated so many mutations that it has lost its viral genes.

Lymphomas and EBV

- In addition to Burkitt's lymphoma, EBV is associated, to varying degrees, with a histologically diverse spectrum of other lymphomas
    - post-transplantation lymphoproliferative disease that develops in environment of T-cell immunosuppression after transplantation
    - Leukemia of NK cell
    - NK/T cell (angiocentric) lymphoma of the nasal type.
- HIV infection increases the risk of developing aggressive B-cell lymphomas and Hodgkin's lymphomas. AIDS patients also develop primary central nervous system lymphoma and rare plasmablastic lymphoma, which almost always contain EBV genes.

Carcinomas and EBV

- In southern China, nasopharyngeal carcinoma (NFC) affects 25 per 100,000 people, accounting for 18% of all cancers in China.
- EBV is present in almost all cases of NFC, both in endemic and non-endemic regions.

- Individuals with rising or relatively high titers of IgA to EBNA1 and capsid antigens are exposed to an increased risk of developing NFC, which may represent a method of early detection of persons in general population with increased risk for tumors development.

- EBV is also present in a small percentage (5% to 15%) of gastric adenocarcinomas and over 90% of gastric lymphoepithelial carcinomas. The prevalence of gastric cancer associated with EBV infection is similar in all regions of the world.

KAPOSHI SARCOMA HERPESVIRUS (KSHV)

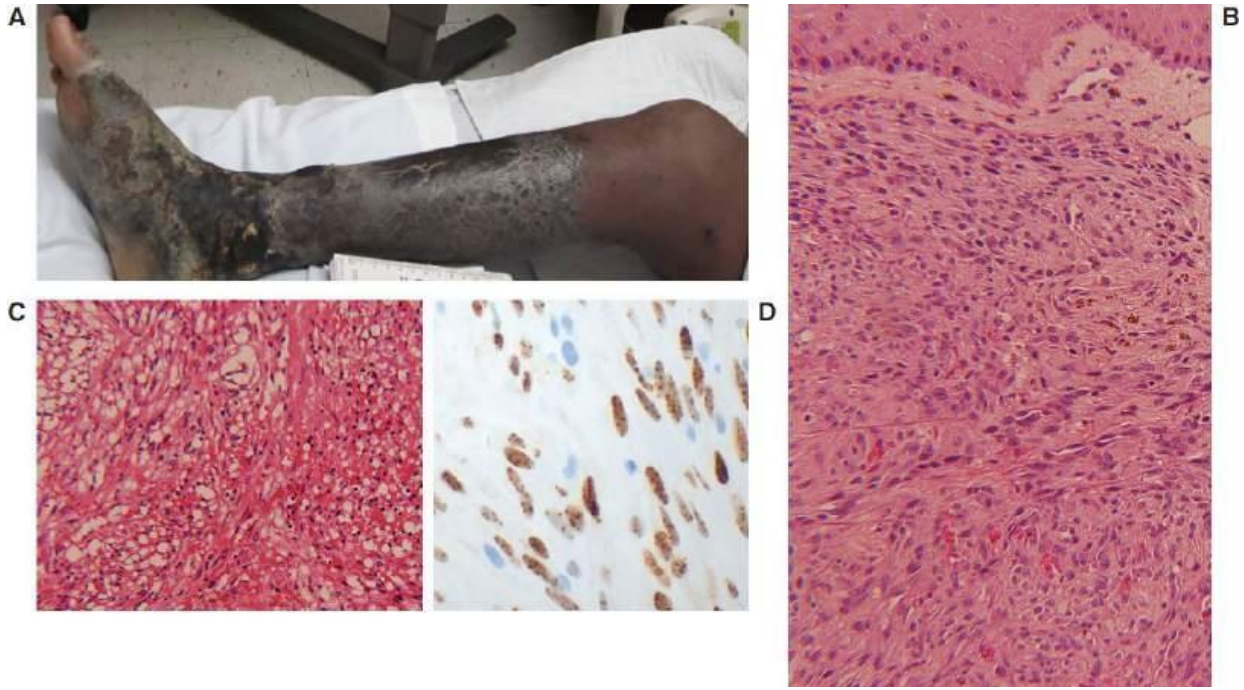
- At the end of the 19th century, Hungarian dermatologist Moritz Kaposi described a relatively rare type of indolent pigmented sarcoma of the skin that affects older men.
- Kaposi's sarcoma (KS) was later found to be more prevalent in the Mediterranean region and in the eastern parts of sub-Saharan Africa.
- Pandemic HIV/AIDS in the 1980s dramatically increased the incidence of highly aggressive forms of KS, particularly among homosexuals who were much younger than typical KS patients, suggesting that the development of KS is associated with the presence of a non-HIV sexually transmitted cofactor.

- Moore and colleagues discovered the presence of a previously unknown herpesvirus in KS tissue in 1994. Kaposi's sarcoma virus, later identified as human herpesvirus-8, was soon shown to be the causative factor for all types of KS, including classic KS in older men, AIDS- related KS (epidemic), transplant-related KS, and endemic KS in sub-Saharan Africa.

- In persons infected with HIV, the risk of developing of KS is inversely proportional to the CD4+ cell count, while control of HIV infection with antiretroviral therapy has reduced the incidence of KS in the United States.

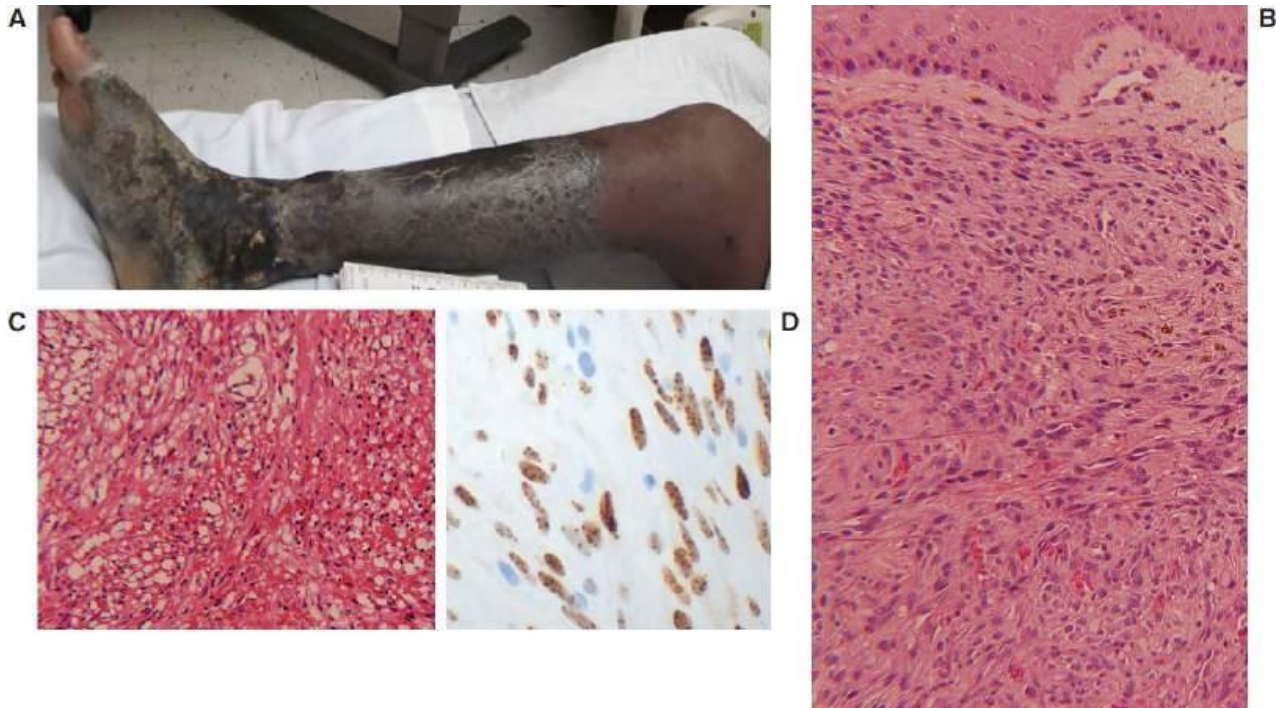
KAPOSHI SARCOMA

- KS is a multicentric tumor, which usually occurs with multiple lesions that do not reflect metastatic disease.
- KS cells infected with Kaposi's sarcoma virus often have a spindle-like morphology and are of endothelium origin, although markers of endothelial cells are not consistently present.
- Endothelial cells infected with Kaposi's sarcoma virus undergo a process of endothelial-mesenchymal transformation, during which they lose endothelial cell markers and begin to express mesenchymal cell markers, which explains the complex phenotype of spindle cells in KS lesions.



KAPOSHI SARCOMA

- KS tumors also contain infiltrating lymphocytes, plasma cells, monocytes, endothelial cells and fibroblasts that are not infected with KSHV, and these additional cell types are believed to provide key signals for tumor cell survival and growth.
- Unlike in EBV infections in which tumorigenesis is driven by the expression of latency-associated genes, the development of KS often appears to depend on the expression of lytic phase genes. This may explain why ganciclovir, which acts on thymidine kinase, can prevent the formation of new KS lesions in HIV-positive individuals.



Lymphoproliferative disorders associated with KSHV

- KSHV causes two forms of B-cell proliferative disorders: multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL).

- MCD occurs more often in AIDS patients and the tumors are almost always positive for KSHV. MCD can occur less frequently in HIV-negative individuals, and in this group only 40% to 50% of cases have KSHV present in the tumor tissue. MCD is a severe systemic disease, characterized by intermittent flares of inflammatory symptoms, including fever, night sweats, and weight loss attributable to acute release of cytokines, particularly IL-6, which may be encoded by KSHV itself.

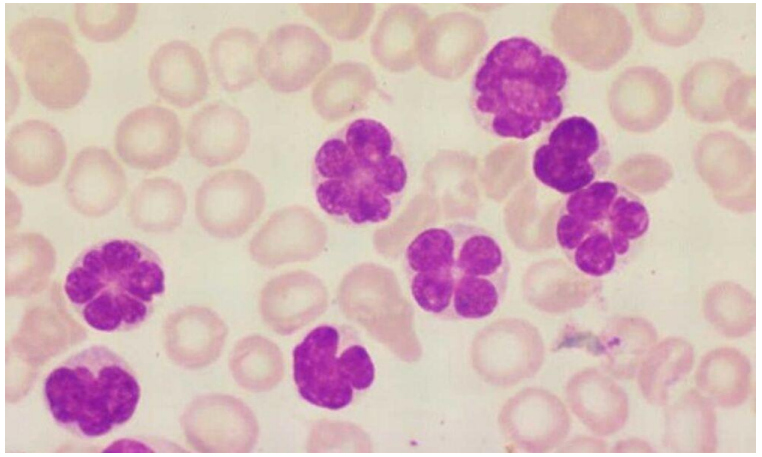
- PEL includes approx. 4% of all HIV-related non-Hodgkin lymphomas. Tumor cells are mostly clonal, they are uniformly infected with KSHV and in 80% of cases they are also infected with EBV. PEL cells have rearranged immunoglobulin genes but do not express markers of mature B cells. They resemble "B1" lymphocytes. Although the origin of PEL cells was uncertain, recent results suggest that KSHV-infected mesothelial cells become PEL tumor cells through the process of transdifferentiation.

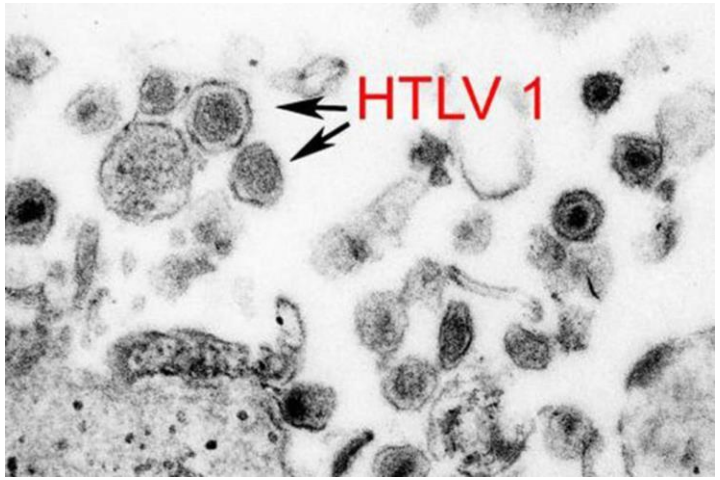
ANIMAL AND HUMAN RETROVIRUSES

- Retroviruses are positive single-stranded RNA viruses that during replication use DNA produced by the transcription of the viral RNA genome.
- There are approx. 100,000 endogenous retroviral elements in the human genome, accounting for nearly 8% of the genetic information, but the potential roles of these elements in disease development are unclear.
- Retroviruses that cause tumors in animals or birds are designated as transforming viruses and can be classified as retroviruses with acute or chronic transformation.
    - Acute transforming retroviruses have acquired a mutated cellular gene, called an oncogene, and cause tumors in animals within a few weeks. Many proto-oncogenes with dominant action in humans (eg, ras, myc, and erbB) were first identified as retroviral transforming oncogenes.
    - Chronic transforming retroviruses integrate into the genome almost randomly and can disrupt the regulation of nearby genes and cause cell proliferation or may suppress apoptosis. Chronic transforming retroviruses cause malignancy many weeks to months after infection. In addition to acute or chronic transformation mechanisms, retroviruses can also transform cells by direct effects mediated by structural or nonstructural viral proteins.

HTLV1

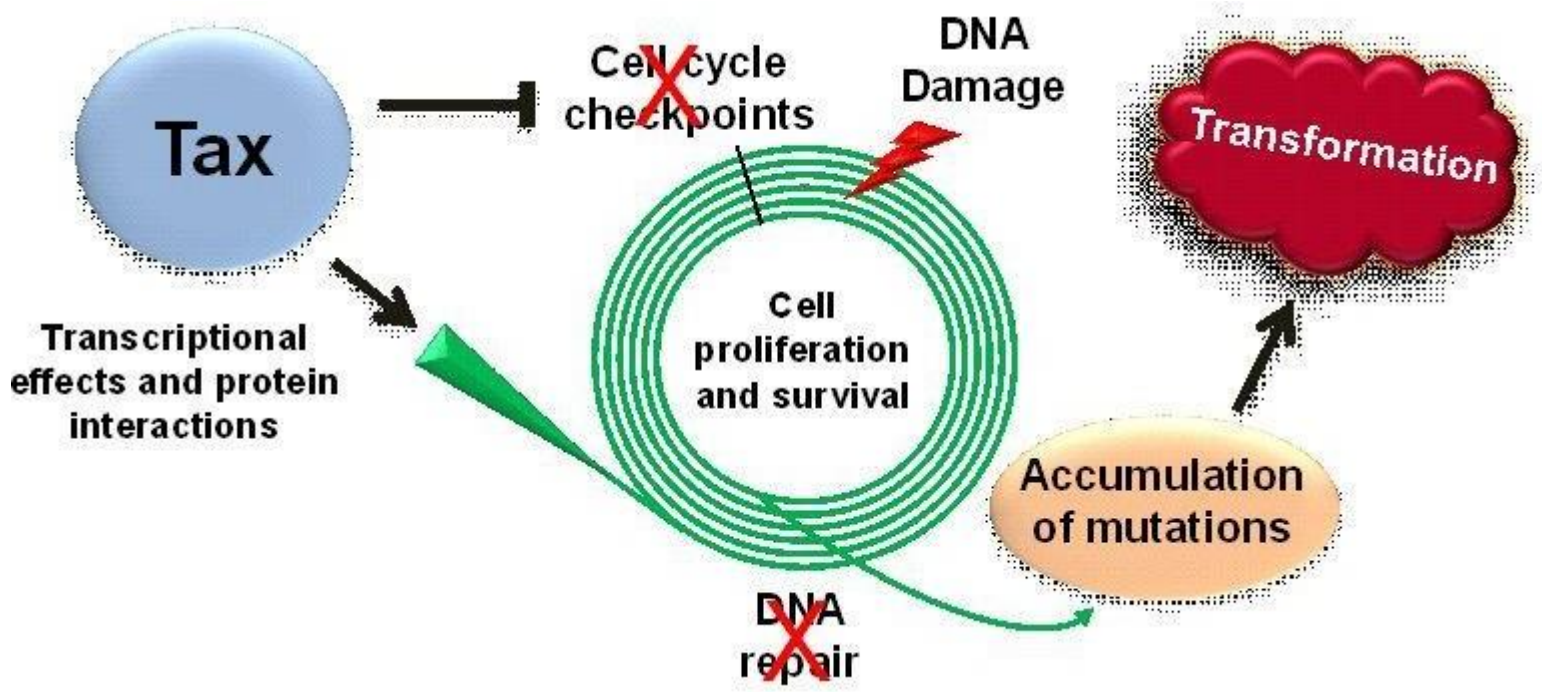
- HTLV1 is the only retrovirus known to be oncogenic for humans and associated with the development of acute T cell leukemia.
- Genes of HTLV1, whose products are responsible for the transformation, encode nonstructural viral proteins that activate signaling pathways in host cells, and the process of cell transformation lasts for years.



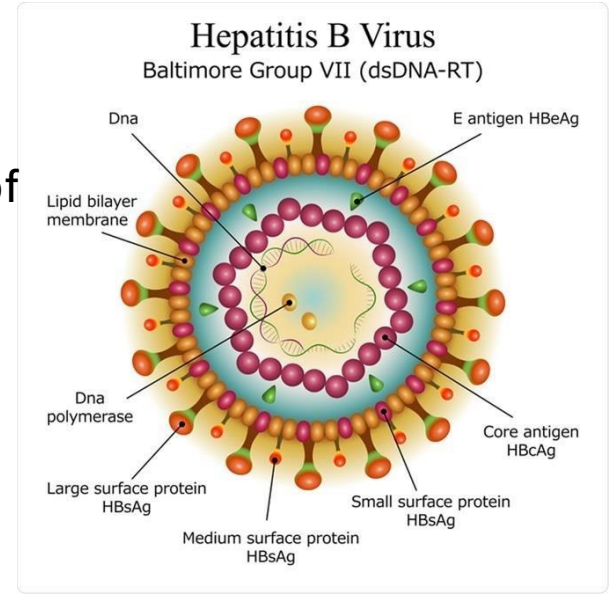


HTLV1

- Viral transcription activatorTax is a protein that can bind different molecules in the cell, by binding to NFkB it promotes cell proliferation and resistance to apoptosis, activates cyclin-dependent kinases and inhibits cell cycle check-point proteins. HTLV1 mainly causes transformation of CD4+ T lymphocytes and is associated with the development of acute T cell leukemia.



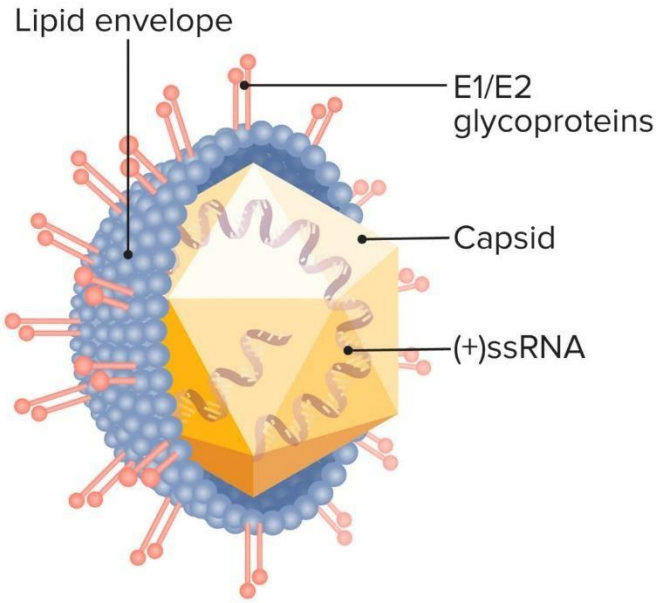
Hepatitis B virus(HBV)



- HBV is an enveloped DNA virus that belongs to the family of *Hepadnaviridae*.
- The viral genome is a circular, partially double-stranded DNA that resides in the nucleus of infected cells where is found in the form of episomes, although chromosomal integration of viral gene sequences can occur during the cycle of regeneration and proliferation of hepatocytes.
- Three main antigens of HBV are HBcAg, HBeAg and HBsAg. Viral protein H (HBh) modulates signal transduction in the host cell.
- After infection, the viral genome is transcribed using host's RNA polymerase II and viral peptides are produced. Nucleocapsids are formed in the cytosol, incorporating a pregenomic RNA molecule into the viral core, where reverse transcription occurs to produce a double-stranded DNA viral genome.

- HBV is not a cytocidal virus. Liver damage occurs as a result of the host's immune response, primarily the response of T lymphocytes and proinflammatory cytokines.
- About 5% of infections in adults and up to 90% of HBV infections in infants result in persistent infection, which may be accompanied by symptoms and elevated serum aminotransferase levels. Immunocompromised people are more likely to have a persistent infection. About 20% of permanently infected people develop cirrhosis.

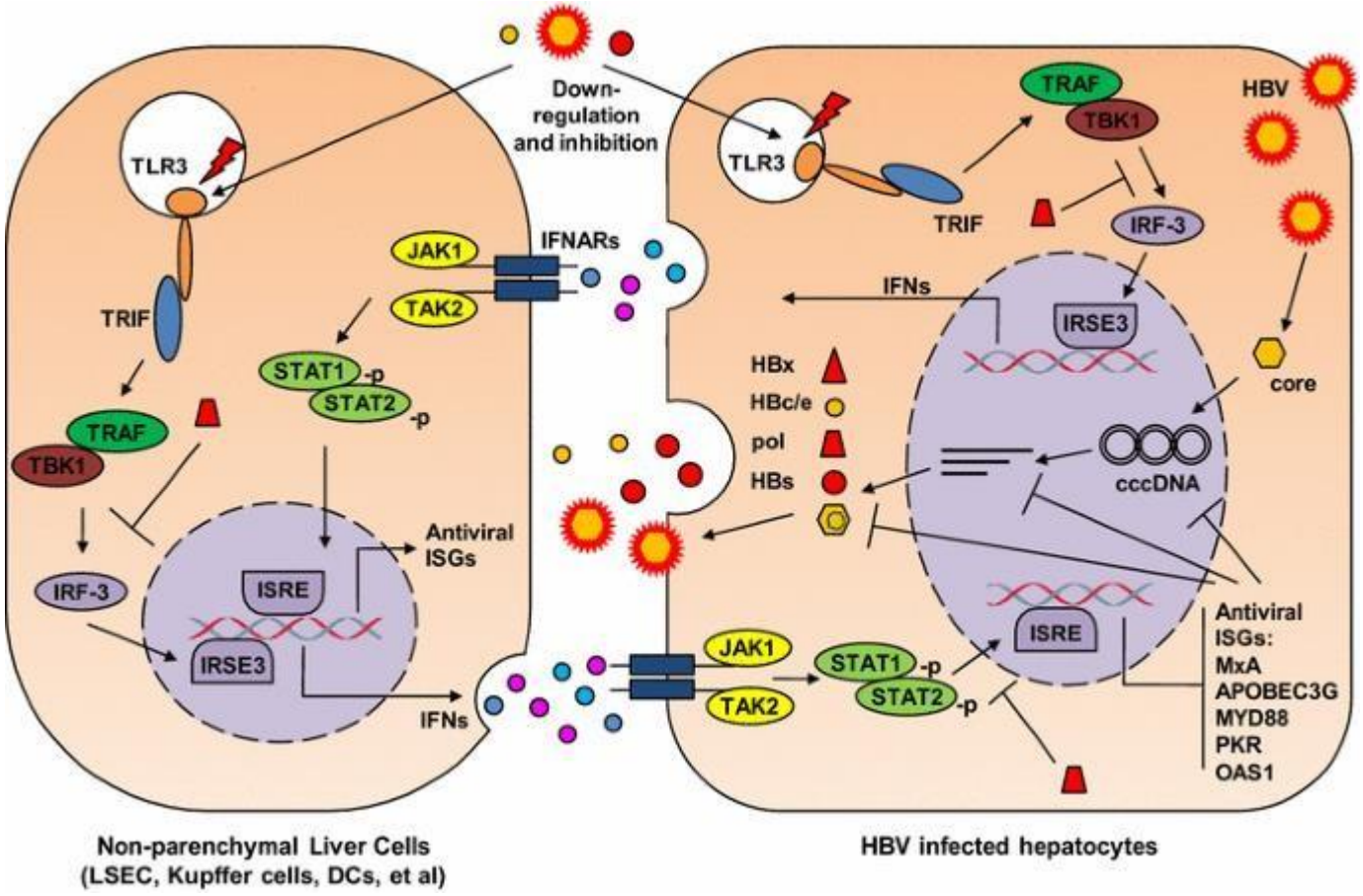
Hepatitis C virus (HCV)



- HCV is an enveloped RNA virus that belongs to the family of *Flaviviridae*.
- HCV replicates in the cytoplasm and does not integrate into the host cell genome.
- In addition to the structural proteins (proteins C)that are included in the composition core, HCV also encodes the nonstructural proteins NS2, NS3, NS4A, NS4B, NS5A, NS5B and p7 that participate in viral replication and assembly.
- Complete elimination of viruses was achieved with the introduction of new therapy in a large percentage of patients, such persons have a reduced risk of developing tumors, but a ten-years follow-up revealed that 2.5% of such persons still have a risk of developing hepatocellular carcinoma (HCC).

Pathogenesis of cell transformation by hepatitis viruses

- HBV and HCV, with proteins, HBx and NS3-4A, suppress the innate immune response by inhibiting TLR receptor signaling.
- HCV inhibits the JAK/STAT signaling cascade, and NS5A and E2 inhibit IFN signaling.
- HBV can also inhibit JAK-STAT signaling but by an unknown mechanism.



Pathogenesis of cell transformation by hepatitis viruses

HBV and HCV induce the development of hepatocellular carcinoma by direct and indirect mechanisms.

- HBx blocks activation of NF-κB pathway, while protein C and NS5A of HCV block apoptosis by activating AKT and NF-κB, respectively.
- Proteins C and NS5 can also induce epithelial-mesenchymal transition, which is important for liver fibrosis.
- HBx and protein C of HCV cause oxidative stress that induces apoptosis. HBs and HBx and NS3-4A alter calcium signaling and increase reactive oxygen species, which triggers endoplasmic reticulum stress and production of proinflammatory cytokines that induce collagen synthesis and fibrosis.
- Both viruses trigger autophagy to restore the integrity of the endoplasmic reticulum, which promotes cell survival and virus persistence.

Pathogenesis of cell transformation by hepatitis viruses

HBV and HCV also disrupt the function of tumor suppressor proteins.

- NS5B of HCV recruits ubiquitin ligase, which modifies pRb and induces its degradation

- ProteinsHBx and the C protein of HCV inhibit the cell cycle inhibitors p16 and p21, which inactivates pRb phosphorylation.
- ProteinsHBx and proteins C, NS3 and NS5A of HCV deregulate the activity of the tumor suppressor r53 thereby compromising p53-mediated DNA repair.
- Both HBV and HSV promote cancer stem cell characteristics. HBx promotes the expression of Nanog, Oct4, Myc. These markers are also induced by hypoxia induced by HBV and HCV infection.