DNA tumor viruses and their role in the development of epithelial tumors

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- Among agents that play an important and often causative role in tumor development there are DNA tumor viruses.

- Especially, human herpesviruses, such as Epstein-Barr virus (EBV), Kaposi sarcoma virus (HHV8), Herpes virus Saimiri (HVS), and Marek's disease virus (MDV) are involved in malignant cell transformation.

- The same is true for small DNA tumor viruses, exemplified by Simian virus 40 (SV40), adenoviruses, and Human papilloma viruses (HPV), though mechanisms of cell transformation are different.

Transformation

- **Changes of the cellular phenotype that makes a "normal" cell acquire features of a "tumor-like" cell**

  in vitro* and/or *in vivo

Types of virus - host cell interaction

- Lytic replication - productive phase (production of 1000s of viral particles from one infecting progeny)
- Latent infection
- Transformation/Immortalization

Integration of viruses into the host cell genome

HPV

- It was shown that viral DNA integrated into the host cell genome in all cases of cervical carcinoma, their metastasis and derivative cell lines.

- Usually DNA is linearized between genes L1 and L2 and integration occurs at the different chromosomes. Regulatory region of HVP genome and E6 and E7 oncogenes remain intact. Other viral genes, such as L1, L2, and E1 are lost or damaged. The transcriptional regulator E2 is inactivated, as a rule. Full length E2 protein prevents entry in S-phase and allows by that the viral replication.

- In result of integration, normal transcripts of E6 and E7 are produced at the elevated level.

- The host cell chromosomal rearrangements play a role in the cancerogenesis. For example, activation of c-myc (8q24) and JUN-B (19p13.2) and deletions on chromosome 3 (3p14, 3q25) contribute to development of tumor.

SV40

- It was shown that if the virus infects the "nonpermissive" cell, viral replication is inhibited in majority of cases.
- In rare cases the viral genome integrates in host genome and transcribed along with the normal cellular genes.
- Large T antigen has the same effect as in permissive cells – it binds to TP53 and RB in the nucleus.
- Such cells can not produce new viral particles, become transformed and lost normal control of the cell growth.

MDV

- In all cell lines, derived from infected T- and B-cells, virus is integrated into genome. Integration is sporadic event and the same integration sites were observed in biopcies (if cell line was derived from biopsy).
- It was debated in the literature, whether the virus can be re-activated from the integrated MDV.
- It was shown also the presence of episomal form of MDV.

HVS

- Herpes virus saimiri exist in the cells usually in episomal form. ORF73 of HVS play the role similar to EBNA-1 and LANA, i.e maintains the viral DNA in episomal form.
- Via 72 amino acids within C-terminus ORF73 binds to MeCP2 (Mehyl CpG binding protein). MeCP2 is a chromosome-associated protein that processes AT-hook domain (as EBNA-1. actually).

HHV8

Episomal form of HHV8 is maintained by help of LANA. LANA binds to MeCP2 and DEK, the chromosome-associated proteins.

EBV

EBV is usually in episomal form in LCLs and BLs.

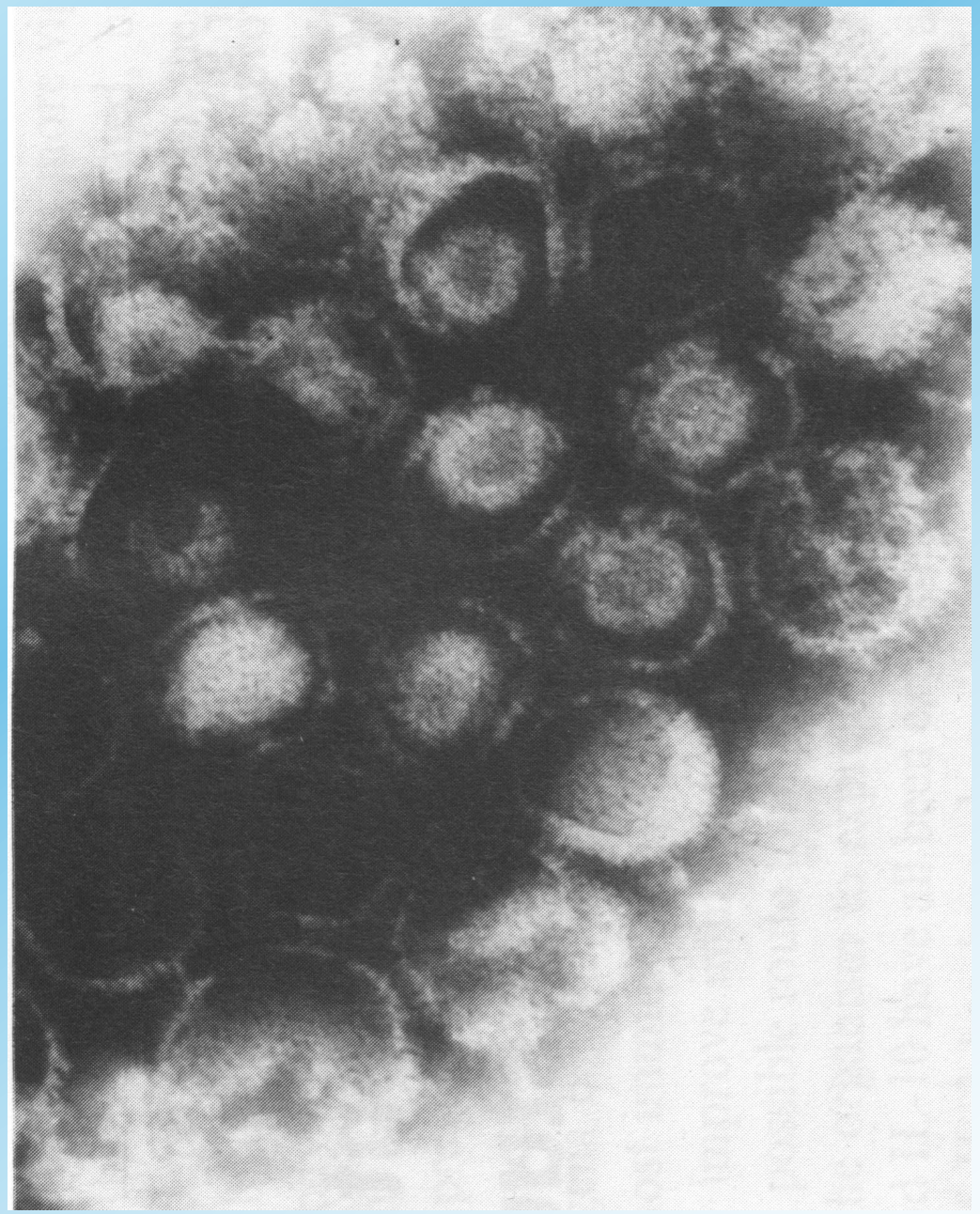
All herpes viruses and small DNA tumor viruses developed mechanisms to inactivate the TP53 and RB pathways

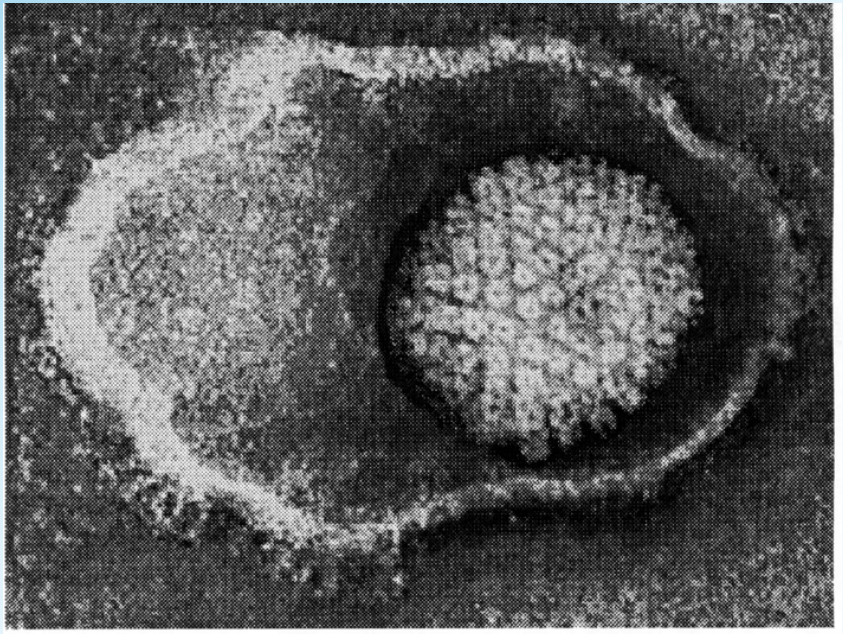
Small DNA tumor viruses

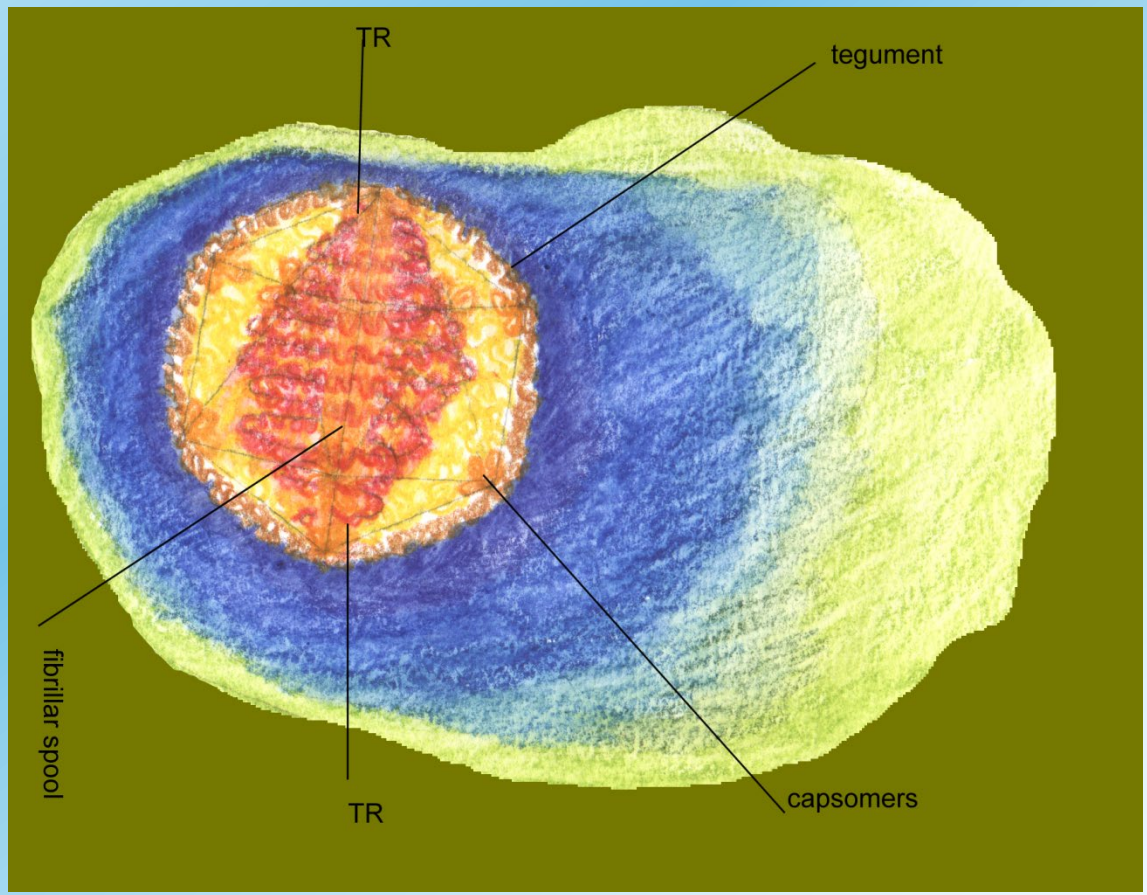
Virus	Usual interaction	p53 pathway			pRb pathway
		p53	p14ARF	MDM2	pRb
SV40	Episomal, rarely integrated in transformed nonpermissive cells.	Large T binds to DNA-binding domain of p53 and abolishes Its function.	Occasional loss of INK4A locus.	Large T can bind MDM2.	Large T binds to pRb (p130, p107) and disrupts pRb-E2F complexes.
Adeno	Integrated in transformed nonpermissive cells.	E1B (55 kDa and 19 kDa) binds to p53. E4orf6 protein binds to p53 and E1B, and targets p53 to degradation.	No data	No data	E1A binds to pRb (p130, p107) and increases free E2F protein level. E1A binds to CtBP.
HPV	Episomal. Integrated in transformed cells of the host.	E6 binds to p53 and targets it to degradation.	Overexpressed in HPV 16/18+ tumors.	Overexpressed in 30% of HPV 16/18+ positive tumors.	E7 binds to pRb and targets it to degradation.

Oncogenic herpesviruses

Virus	Usual interaction	p53 pathway			pRb pathway
		p53	p14ARF	MDM2	pRb
EBV	Episomal, rarely integrated in transformed cells.	No data	EBNA-5 binds to p14ARF.	EBNA-5 binds to MDM2.	EBNA-3 and EBNA-6 bind to CtBP and repress RBP-J-kappa-dependent transactivation. EBNA-6 binds to and raises the level of nuclear MRS18-2, a pRb-binding protein. That inhibits pRb binding to E2F and repressor function.
HHV8	Episomal	LANA binds to p53	No data	No data	LANA binds to pRb; v-cyclin p-ylates pRb and prevents E2F binding
HVS	Episomal	ORF73 product binds to p53	No data	No data	ORF73 product binds to pRb; v-cyclin p-ylates pRb and prevents E2F binding
MDV	Integrated in transformed cells, could be also episomal	Meq transactivates bcl-2	No data	No data	Meq binds to cdk2 and CtBP







200 nm

Enveloped

Double-stranded linear DNA

172.274 bp

82 open reading frames

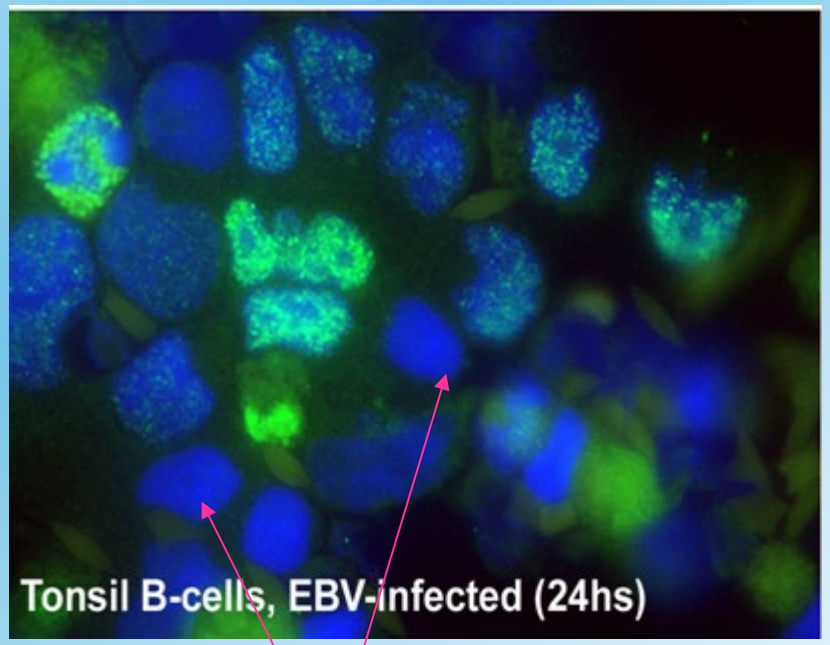
12 genes used in control of latency

71 genes used in virus replication

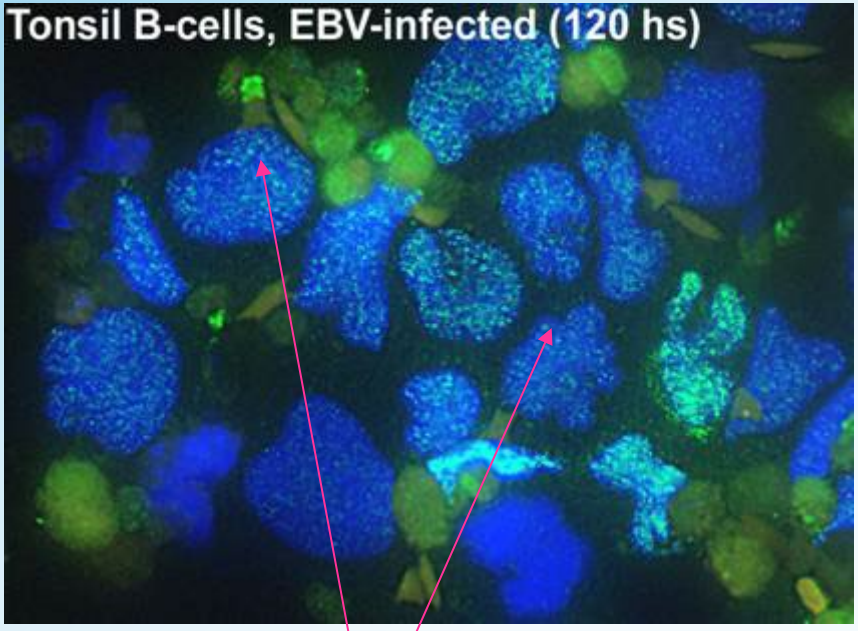
Life style of Epstein-Barr virus (EBV)

Latent infection	Lytic (productive) infection
In B lymphocytes	In epithelial/B cells
Latent programs I-III	
12 genes	80 genes

Latent EBV infection of resting B cells converts them into transformed cells that can develop into tumors in immuno-compromised hosts



B-cells



Lymphoblasts

EBV infection is a unique, well-defined *in vitro* system for malignant transformation.

The paradox

Most common virus in the human population (>90% of all adults, life long persistence)

Tumor-associated virus

80% of carriers secrete biologically active virus (saliva)

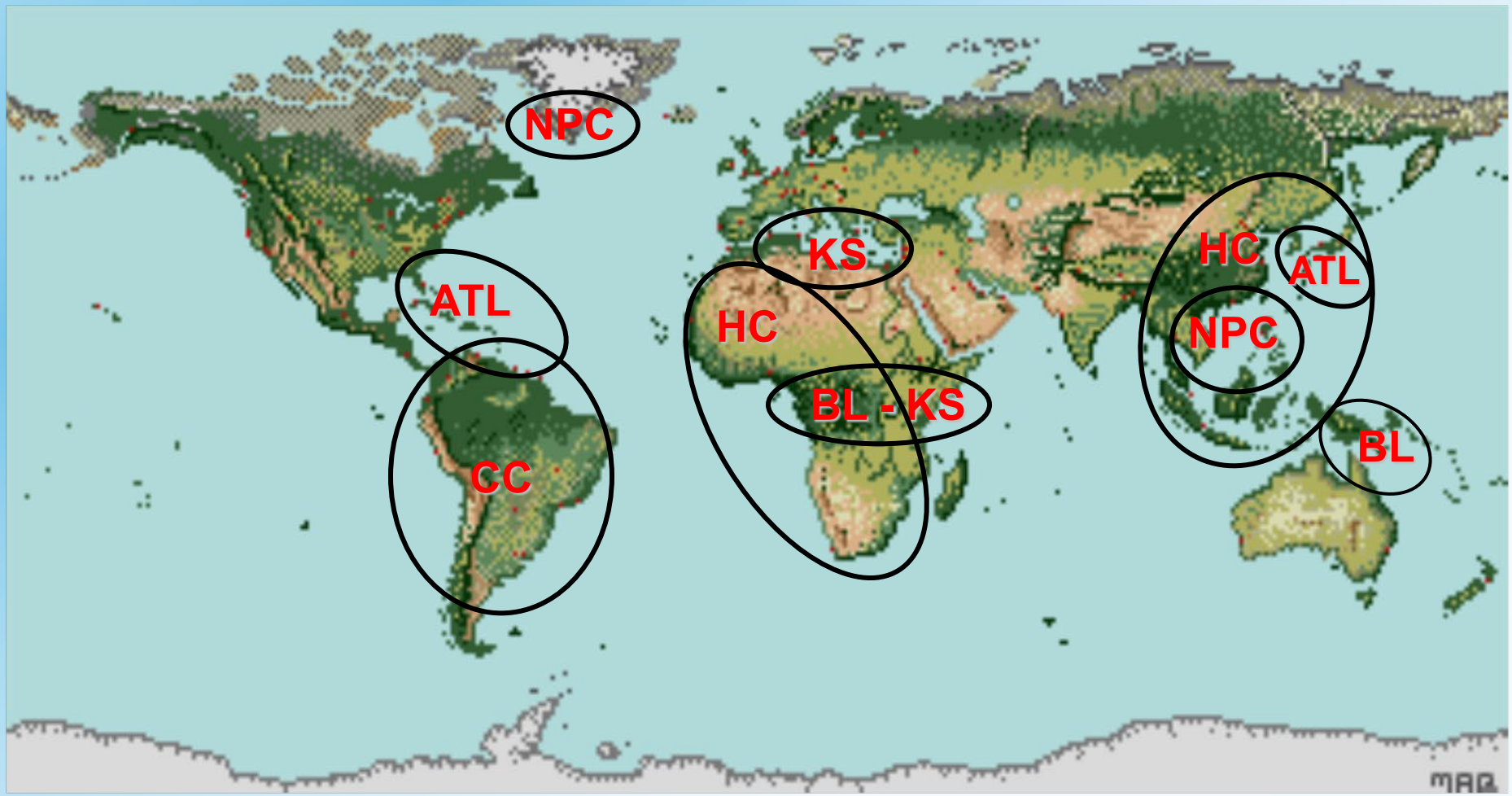
Very efficient immortalizing B-cells

EBV is the most prevalent virus

170,000 new cases of cancer/year

• Nasopharyngeal carcinoma (NPC)	58,000	100%
• Stomach cancer	50,000	5-10%
• Hodgkin lymphoma	30,000	40-50%
• T-cell lymphoma	20,000	30-40%
• Non Hodgkin lymphomas in AIDS	6,000	25-100%
• Burkitts lymphoma	3,000	98%
• Post-transplant lymphomas	500	99%

Geographic distribution of virus-associated tumors



Epstein-Barr virus

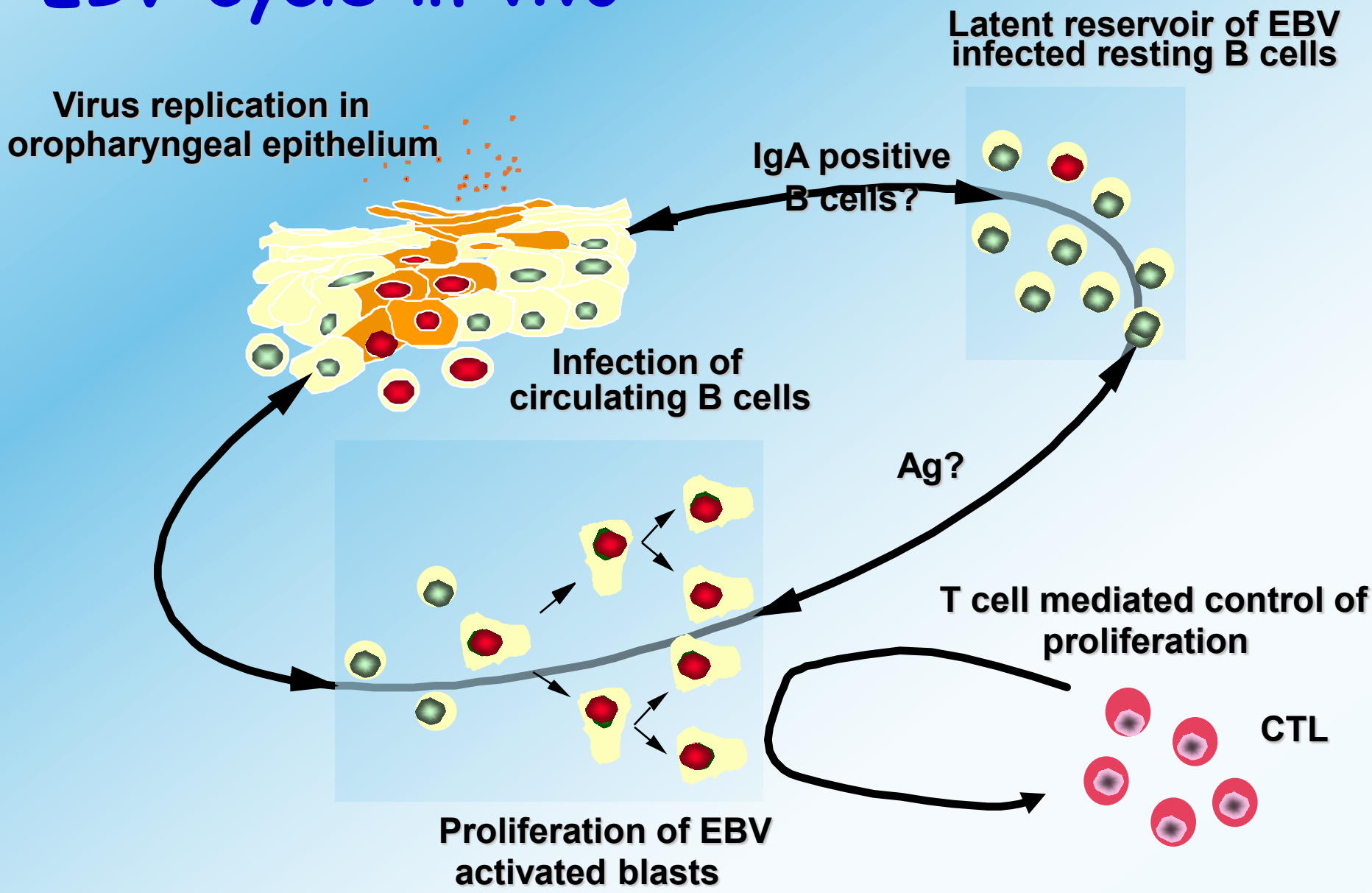
The accidents:

Combined immunosuppression, immunodysregulation and chronic antigen stimulation

Infection of the wrong cell type: T-cells, smooth muscle, epithelial cells?

Infection in unfavourable genetic background (chinese, inuits, X-linked lymphoproliferative syndrome, ataxia-telangectasia)

EBV cycle in vivo



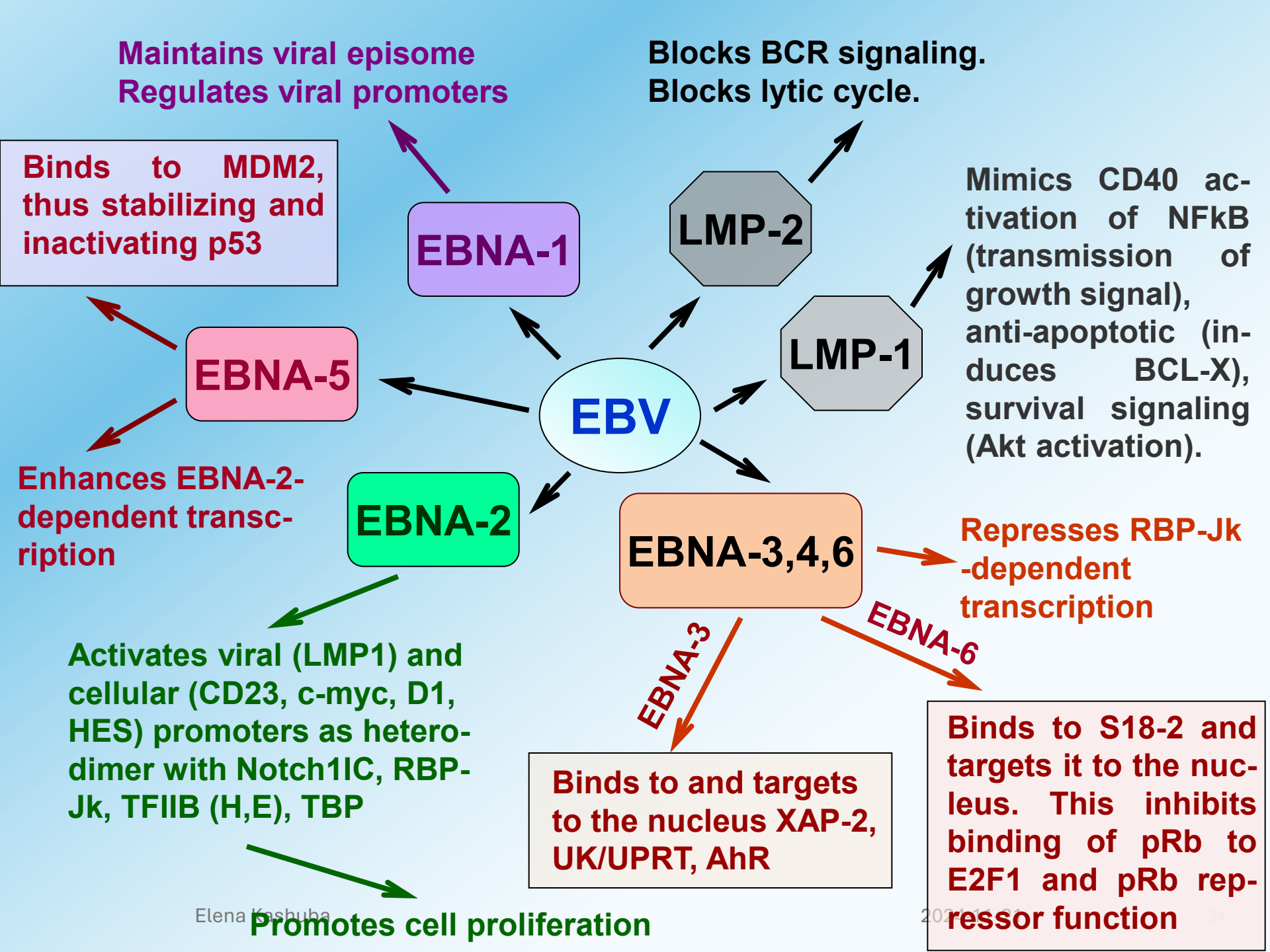
Programs of EBV infection in latency and tumors: gene expression pattern

Latency I	EBER 1 & 2 EBNA 1	GC, NPC (35%), BL, healthy carriers (similar)
Latency IIA	EBER 1 & 2 EBNA 1, LMP1	NPC (65%)
Latency IIB	EBER 1 & 2 EBNA 1, LMP1	CLL *in vitro*, HL
Latency III	EBER 1 & 2, EBNA 1-6 LMP 1, LMP 2a & b	Immunoblastic, Post-transplant&AIDS lymphomas, Primary infection (IM)

Transformation related functions	
EBNA-1	Maintains viral episome. Regulates viral promoters.
EBNA-2	Activates viral (LMP1) and cellular promoters as heterodimer with Notch1IC, RBP-Jκ, TFIIB (H,E), TBP. Promotes cell proliferation.
EBNA-3	Binds to and targets to the nucleus XAP-2, UK/UPRT, AhR. Represses RBP-Jκ-dependent transcription.
EBNA-4	Represses RBP-Jκ-dependent transcription.
EBNA-5	Enhances EBNA-2-dependent transcription. Binds to p14ARF and MDM2, thus targeting p53 pathway.
EBNA-6	Binds to and raises the level of nuclear MRS18-2, a pRb-binding protein, thereby inhibits pRb binding to E2F and repressor function. Represses RBP-Jκ-dependent transcription.

Transformation related functions	
LMP1	Mimics CD40 activation of NFκB (transmission of growth signal), anti-apoptotic (induces BCL-X), survival signaling (Akt activation).
LMP2	Interacts with phosphotyrosine kinases (src-family, PI3). Blocks lytic cycle. Rescues surface Ig negative B cells.

B-cell specific functions	
EBNA-1	Not known.
EBNA-2	Interaction with PU.1 (Spi1), expression of giant mRNA. Expressed only in B-cells.
EBNA-3	Expressed only in B-cells.
EBNA-4	Expressed only in B-cells.
EBNA-5	Expressed only in B-cells.
EBNA-6	Expressed only in B-cells.
LMP1	CD40-like activation.
LMP2	Blocks BCR-signaling. Promotes survival of B-cells.


Maintains viral episome
Regulates viral promoters
Blocks BCR signaling.
Blocks lytic cycle.
Binds to MDM2, thus stabilizing and inactivating p53
EBNA-1
LMP-2
Mimics CD40 activation of NFkB (transmission of growth signal), anti-apoptotic (induces BCL-X), survival signaling (Akt activation).
EBNA-5
LMP-1
EBV
Enhances EBNA-2-dependent transcription
EBNA-2
EBNA-3,4,6
Represses RBP-Jk -dependent transcription
EBNA-3
EBNA-6
Activates viral (LMP1) and cellular (CD23, c-myc, D1, HES) promoters as heterodimer with Notch1IC, RBP-Jk, TFIIB (H,E), TBP
Binds to and targets to the nucleus XAP-2, UK/UPRT, AhR
Binds to S18-2 and targets it to the nucleus. This inhibits binding of pRb to E2F1 and pRb repressor function
Promotes cell proliferation

EBNA binding proteins

EBNA-3	RBP-Jκ; RBP-2N; CtBP; TCP-1; XAP-2; uridine-cytidine kinase 1-like 1; AhR (DR); VDR; H2A; H2B; SWI/SNF1; HIF1A; PHD2; chk2/cds1 kinase
EBNA-5	Hsp27; Hsp70 (Hsp72); Hsc70 (Hsp73); HAX-1; HA95; α and β tubulins; prolyl-4-hydroxylase α-1 subunit; p14ARF; Fte-1/S3a; MDM2; PHD1
EBNA-6	RBP-Jκ; RBP-2N; DP103; ProT- α; SMN; NM23-H1; HDAC1; CtBP; PU.1 (Spi 1), Spi-B; Skp2; Roc-1; p50 of NFκB; c-fos; Sp1; Cyclin A; MRS18-2 (RB binding protein)

We have identified EBNA-binding proteins that are involved in:

- cell metabolism (**PHD1, PHD2, HIF1α, UCKL-1**),
- cell cycle control, regulated by tumor suppressor proteins p53 (**p14ARF, MDM2**) and RB (**MRPS18-2**),
- nuclear receptor signaling cascades (**XAP-2, AhR, VDR, HIF1α**),
- control of apoptosis (**MDM2, AhR, VDR**),
- cell transformation (**S3a, MRS18-2**),
- protein folding (**TCP-1**).

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