British Microbiology Research Journal
3(2): 176-189, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Genetic Instability and Herpes Simplex Virus

H. S. Zakaryan[1] and Z. A. Karalyan[1*]

[1]Laboratory of cell biology, Institute of Molecular Biology of NAS, 0014, Yerevan, Armenia.

Authors' contributions

This work was carried out in collaboration between both authors. Author HSZ wrote the first draft of the manuscript. Author ZAK planned and revised the manuscript. Both authors read and approved the final version of manuscript.

Review article

Received 8th December 2012
Accepted 5th March 2013
Published 22nd March 2013

ABSTRACT

Although inhibitory mechanisms that safeguard cells against DNA damages occur in all cells, genetic instability is widely present throughout living world. Some well known viruses cause alterations in host genome, leading to the formation of malignant transformations. Throughout this review we discuss some forms of instabilities caused by herpes simplex virus. Herpes simplex virus may induce chromosomal instabilities by various mechanisms, such as by formation of syncytium or ICP0-induced degradation of constitutive centromere proteins as well as may induce accumulation of point mutations by means of oxidative stress in neurons. We believe that further investigation of the ability of herpes simplex virus to cause genetic instability may help us to increase our understanding about the nature of this phenomenon.

Keywords: Aneuploidy; herpes simplex virus; multinucleate cell; polyploidy.

1. INTRODUCTION TO GENETIC INSTABILITY

During billion years of evolution cells have evolved the mechanisms that safeguard their own genome against exogenous and endogenous sources of DNA damage and ensure accurate passage of genetic information onto daughter cells. Although cells have mechanisms (intricate machinery of repair, damage tolerance, and checkpoint pathways) preventing mutations and DNA rearrangements, genetic instability is widely present throughout the living world, from bacteria and single cell organisms to multicellular beings. In spite of the

**Corresponding author: Email: z_karalyan@mb.sci.am;*

harmful influence for the cell and subsequently the whole organism, genetic instability contributes to adaptation at the molecular level and propagates genetic diversity. Genetic instability also involved in the generation of variability in the developmental processes, such as immunoglobulin diversification [1]. Nonetheless, genetic instability is usually associated with pathological upsets, and in humans it is associated with premature ageing [2], with inherited diseases (such as mental disorders and retardations) and with various types of cancer.

Genetic instability is a fundamental hallmark of almost all human cancers. It refers to a range of genetic alterations from chromosomal rearrangements to point mutations that are similar for both exogenous and endogenous mutagens and occur in all types of cancer. There are various forms of genetic instability. Chromosomal instability refers to changes in chromosome number that lead to chromosome loss or augmentation, and it is usually caused by failures in chromosome segregation or spindle checkpoint (Table1) [3]. Micro- and minisatellite instability leads to shrinkages or expansions of short DNA repeat sequences and can occur by homologous recombination, by mismatch repair errors and by replicative fork slippage [4]. Most hereditary alterations in the human genome encompass point mutations, including micro-insertions, micro-deletions and nucleotide substitutions, and those instabilities are mainly associated with replication errors, impairment of mismatch repair [5] and base excision repair [6].

Table 1. Classification of genetic instable based on changes of DNA content

Polyploidy - multiplication by diploid set of DNA content	Multinuclear cells	Symplast	Multinuclear cells formed by rounds of i***ncomplete mitosis (absence of cytokinesis)***
		Syncytia	Cells-formed by the fusion of mononucleated cells with the disappearance of intercellular plasma membrane
	Polyploid nuclei	Type1	Formed by fusion of nuclei of multinuclear cells
		Type 2	Formed by rounds of i***ncomplete mitosis***
		Polythene	Formed by rounds of i***ncomplete cell cycle***
Aneuploidy - any deviation in DNA content from polyploidy state	Abnormal number of chromosomes	Heteroploid nuclei	Formed by mitotic mistakes
	Chromosomal instability	Deletions Duplications Translocations	Formed as a result of different mutations occurred during mitosis or cell cycle
	Gene amplification		Production of many copies of gene or genes

Genetic instability has been reviewed extensively elsewhere and many works have been dedicated to complete examination of various types of genetic instability and their role in cancer rise and further progression [7]. In this review we discuss how viruses (particularly herpes simplex virus) lead to various types of genetic instability during their acute or chronic infections.

2. VIRUS-INDUCED GENETIC INSTABILITY

The discovery of Epstein-Barr virus in cells from Burkitt's lymphoma in 1964 [8], hepatitis B virus in human sera positive for hepatitis B surface antigen in 1970 [9] and human papillomavirus 16 and 18 from human cervical cancer specimens in 1983 and 1984 respectively [10,11], restored interest in the roles of viruses in genetic instability and human cancer. After 50 years of exciting discoveries, it has become extremely apparent that several viruses have been etiologically linked to specific forms of cancer and many other viruses encode proteins that are implicated in genetic instability. Viruses are one of the main cancer risk factors in humans, and several cancers are directly associated with viral infections (Table 2) [12].

Table 2. Directly carcinogenic human viruses

Virus	Virus Family	Genome	Cell Tropism	Human Cancers
DNA viruses				
HBV	Hepadnoviridae	dsDNA 3.2kb	Hepatocytes, white blood cells	Hepatocellular carcinoma
EBV	Herpesviridae	dsDNA 172kb	Epithelial cells, B-cells	Lymphoma, nasopharyngeal carcinoma
HPV	Papillomaviridae	dsDNA 8kb	Squamous epithelial cells	Cervical, oral and anogenital cancer
KSHV	Herpesviridae	dsDNA 165kb	B-cells	Kaposi sarcoma, lymphoma
RNA viruses				
HCV	Flaviviridae	dsRNA 9.4kb	Hepatocytes	Hepatocellular carcinoma
HTLV	Retroviridae	dsRNA 9.0kb	T-cells	Adult T-cell leukemia

HBV, hepatitis B virus; EBV, Epstein-Barr virus; HPV, human papillomavirus; KHSV, Kaposi's sarcoma-associated herpesvirus; HCV, hepatitis C virus; HTLV, human T-cell leukemia virus

DNA tumor viruses are a diverse group with varied structures, genome organizations, and replication strategies. Some DNA tumor viruses may directly influence on host genomic stability, whereas others impact on mechanisms that prevent cells from instability. For example, hepatitis B virus integrates own genome into the host chromosome (for persistence of viral genome [13]), and it can result in chromosomal deletions and transpositions of viral sequences from one chromosome to another [14]. In other words, hepatitis B virus integration leads to genetic instability [15] and this event may precede tumor development [16]. On the other hand human papillomavirus E6 protein targets Bak (proapoptotic Bcl-2 family member) for degradation. Bak plays an important role in signaling apoptosis in response to UV irradiation. Also, E6 protein associates with and dysregulates cellular regulatory protein complexes, especially p53 and pRB that control cellular proliferation, differentiation and apoptosis. Cells that are infected by human papillomaviruses are less

prone to undergo apoptosis after UV induced DNA damages [17], have a decreased ability to maintain genomic integrity [18] and contain extensive chromosome aberrations.

In contrast to the well studied DNA tumorigenic viruses, the role of RNA viruses in genetic instability and carcinogenesis is poorly investigated. At present, only retroviruses have been associated with genetic instability. It is well known that after infection the retroviral RNA genome is reverse-transcribed by the virally encoded reverse transcriptase into a double-stranded DNA copy which then integrates into the host chromosome. As a consequence of integration, retroviruses reorganize cellular genetic material (which is a classical example of genetic instability) and control host genes expression. Though the biological impact is still poorly understood, extensive studies of retroviruses led to the discovery of proto-oncogenes that have crucial roles in the control of cell growth and differentiation [19,20]. The discovery of proto-oncogenes has become a turning point in our understanding of mechanisms of carcinogenesis.

3. HERPES SIMPLEX VIRUS AND GENETIC INSTABILITY

Herpes simplex virus (HSV) belongs to the family of *Herpesviridae*. HSV has two serotypes, HSV-1 and HSV-2, whose formal designation according to the rules of the International Committee on Taxonomy of Viruses are human herpesviruses 1 and 2 (through this review we use informal designation). Although their size and complexity served as obstacles to intensive research for many years, now HSV is among the most intensively studied of all viruses. The attractiveness of HSV is a result of distinct biologic properties that is possesses, particularly the ability to cause a variety of infections, remain latent in their host and reactivate to cause lesions near the sites of infection.

The genome of HSV is double-stranded, linear DNA, which becomes circular in the absence of protein synthesis after entry to the nuclei of infected cells [21]. The nuclei of cells are the only place where HSV replication takes place. Knipe and colleagues [22] showed that effective viral infection follows from the remodeling of the nucleus of HSV-infected cells because remodeling provides the structural basis for viral DNA replication and gene expression [23]. Major alterations of nuclear structure may lead to genetic instability in host cells. Below we discuss some forms of instabilities caused by lytic or chronic HSV infection.

3.1 Abnormal Chromosome Set due to the Formation of Syncytium

The syncytium, as a distinct phenomenon, is usually referred to the genetic instability due to abnormal chromosome set in a single multinucleate cell [24]. The formation of multinucleate cells, or syncytium, results from destruction of cell walls and fusion of single nuclear cells into a single cell mass with two or more nuclei [24]. The fusion of cells seems to be the main mechanism that is responsible for the formation of syncytium both *in vivo* [25] and *in vitro* [26,27].

Viruses, such as HSV, that replicate in the nucleus could benefit from the presence of additional nuclei within a syncytium. The ability of HSV to cause the formation of syncytium was shown by Roizman in 1962 [28]. At present, it has been published that a few numbers of viral glycoproteins and cell receptors are involved in HSV-induced cell fusion, but a precise mechanism that leads to fusion remains unclear. It is believed that HSV-induced cell fusion is a complex process that involves the concerted and subsequent actions of multiple viral glycoproteins and membrane proteins. The glycoproteins gD, gB, gH and gL form a

functional complex that is required for both virus entry [29] and virus-induced cell fusion [30]. The binding of gD to its cognate host receptors, herpesvirus entry mediator (HVEM) or nectin-1, probably triggers conformational and possibly sequential changes to gH/gL heterodimer and then gB to promote membrane fusion [31,32,33]. Tiwari and colleagues [34,35] showed that the formation of syncytium heavily depends not only on HVEM or nectin-1 interaction with glycoproteins but also on expression of 3-O-sulfated heparin sulfate, a well known receptor for HSV attachment [36] and penetration [37], on targets cells. Although HSV-induced formation of syncytium plays an important role in the cytopathology of HSV, there is a huge gap in our knowledge about accurate mechanisms of syncytium formation, and further studies may fill the gap. In any case polykaryocyte formation is an important means of viral spread (cell-to-cell spread by polykaryocyte formation) and a clue for HSV-1 keratitis [35].

3.2 Abnormal Chromosome Set: Polyploidy

Polyploidy, a condition in which a normally diploid cell acquires one or more additional sets of chromosomes, occurs frequently in nature. Polyploid cells can arise by a variety of mechanisms, including cytokinesis failure [38], mitotic slippage [39], virus-induced cells and nuclear fusion [40] etc. The influence of HSV on cell cycle control leading to deviation of cell cycle and polyploid cell formation was discussed many years ago in some articles [41]. The hypothesis arose among members of the HSV community that a few viral proteins like ICP4 (infected cell protein 4), ICP8 or DNA polymerase may be involved in mitotic arrest and subsequently in polyploid cell formation. Further studies examined the influence of another viral protein, ICP0, whose leading role in mitotic arrest have been demonstrated by two different research groups [42,43]. They showed that ICP0 can destruct CENP-C thereby arresting cells in G2/M phase. According to recent studies ICP0 disassembles microtubules of infected cells which may also lead to cell arrest [44]. In both cases, HSV uses a pathway that is fundamentally different from the inactivation of p53 by the HPV E6 protein for ICP0-mediated G2/M arrest, utilizing Chk2, a protein kinase that is usually activated in response to DNA damages [45].

In addition to ICP0-mediated cell arrest we can suggest two alternative mechanisms that may be implicated in polyploid cell formation. Love and Wildy in 1963 reported that the first detectable abnormality in HSV-infected HeLa cells was an enlargement of RNA containing cellular organelle, nucleolini, followed by their extrusion from the nucleolus into the nucleoplasm [46]. The role of nucleolini in cell cycle was discovered by Alliegro and colleagues who showed that normal spindle formation is tightly correlated with location of nucleolini in nucleolus [47]. These findings suggest that the direct influence of HSV on location of nucleolini may result in deviation of cell cycle and polyploidy formation.

The second proposed mechanism based on our studies (unpublished) is HSV-induced nuclei fusion in syncytium. We observed the formation of giant nuclei in SK-N-MC cells after a short time of HSV infection (10-12 hours). The giant nuclei were characterized by defective chromatin structure, absence of nucleoli and up to 90c (c-value refers to the amount of DNA contained within a haploid nucleus) DNA content. The formation of giant nuclei after such a short time of infection can be the consequence of virus-induced nuclei fusion. We can speculate that some viral glycoproteins are involved in nuclear fusion in the same way as they are responsible for the fusion of the viral envelope with the outer nuclear membrane [48]. However, this phenomenon requires further investigation.

3.3 Abnormal Chromosome Set: Aneuploidy

Aneuploidy is one sort of chromosome abnormality characterized by missing a chromosome or having extra copies. A frequent characteristic of cancer cells, aneuploidy generally occurs during cell division when the chromosomes do not separate correctly between the two newly forming daughter cells [49]. Chromosomal rupture around the centromeric region and a poorly controlled mitotic spindle are main mechanisms leading to aneuploidy [50].

HSV infection of cultured cells induces the formation of aneuploidy by two different ways. As previously mentioned, ICP0 plays an important role in HSV-induced chromosomal instability due to destruction of essential component of centromere. ICP0 is a RING finger nuclear protein with characterized E3 ubiquitin ligase activity [51]. As soon as ICP0 enters the nucleus, it provisionally localizes to centromeres and induces the proteasomal degradation of centromere proteins [42,52,53]. Therefore, ICP0-induced degradation of constitutive centromere proteins is likely to modify the structure of the central core region, thereby preventing the assembly of the kinetochore. Chromosomes frequently fail to align at a compact metaphase plate when CENP-C is targeted by ICP0 [42], leading to lost of accurate chromosomal segregation and aneuploidy.

The amplification of only some parts of the genome usually results in formation of genetic instability, particularly aneuploidy [54]. Although HSV suppresses the ability of the host to replicate [55], it may induce amplification of small-sized parts of the host genome. Some experiments show that the replication machinery of HSV can be involved in the amplification of other viral genomes like papillomavirus or simian virus 40 [56,57]. The importance of these findings is that HSV may amplify part of the DNA that does not belong to the host genome. Other studies demonstrate that viral polymerase holoenzyme (UL30/UL42) as well as helicase-primase heterotrimer (UL5/UL8/UL52), origin binding protein (UL9) and single-stranded DNA binding protein (UL29) are implicated in the amplification of host genome [58,59].

Galloway and McDougall [60] showed that HSV is able to cause mutations-both point mutations and gene rearrangements. HSV is also able to induce gene amplification, particularly of sequences harboring an origin of replication such as SV40 or papillomaviruses. Other experiments have shown that HSV can activate the expression of endogenous retroviruses.

3.4 Chromosome Structural Abnormalities

One of the consequences of a viral infection of mammalian cells is chromosome structural damage. Many DNA and RNA viruses induce a wide range of aberrations *in vitro* and *in vivo* [61]. The ability of HSV to cause chromosome structural abnormalities was reported many times during the 1960s [62,63,64]. For example, Hampar and Elison demonstrated that HSV infection forms multiple chromosomal aberrations in Chinese hamster cells, especially deletions that occur at a frequency of 80-100 per cent in all infected cells [65]. Different types of translocations and non-homologue recombination are also detected during HSV infection, including translocations looking like robertsonian type, as almost all infected cells have a new chromosome which would not be identified, and is morphologically different from cell to cell [66,67]. HSV may induce uncoiling of chromosomes near the lq12-21 and to a lesser extent the pericentric regions of chromosomes 1, 9 and 16 [68].

How does HSV induce such a variety of chromosome structural abnormalities? The accurate mechanisms involved in the formation of chromosome abnormalities in general are still poorly investigated. At least three theories – telomere attrition, defects in double-strand break (DSB) repair and fragile sites – have been suggested as structural abnormalities [50]. Errors in mitotic chromosome segregation may generate DNA breaks via the formation of structures called micronuclei. It has been shown that ICP0 induces centromeric disruption and appearance of micronuclei [69]. The centromeric protein CENP-C was lost from centromeres during virus infection in an ICP0- and proteasome-dependent manner, causing substantial ultrastructural changes in the kinetochore. This results in not only cellular arrest in the G2/M phase (shown above), but also an unusual cytokinesis with many micronuclei [42].

Improper DSB repair may lead to chromosomal rearrangements, such as deletions or translocations, detected during HSV infection, because of continuous *de novo* generation of breaks [70,71]. HSV-infected cells exhibit high frequency of improper DSB repair, which means that the virus may influence host repair mechanisms, leading to the accumulation of chromosomal rearrangements [72,73]. On the other hand, HSV can induce DSBs by means of the reactivation of some cellular proteins, such as endonuclease G. Endonuclease G is normally localized in mitochondria and released from them during apoptosis, inducing DNA fragmentation [74]. HSV requires endonuclease G in abundance for successful infection, and therefore it is recruited from mitochondria and translocated in nucleus, implicating in initiation of genomic inversion of HSV and formation of DSB in host DNA [75].

3.5 Does HSV Induce Gene Mutations?

After the pioneering work of Schlehofer and zur Hausen [76], reporting on the induction of mutations in the host genome by HSV-1, many scientists from the HSV community tried to identify the mutagenic role of the virus on host genes. In spite of extensive investigation, only few studies identified the mutagenic influence of HSV on host genes [77,78]. Conversely, there are striking evidences that virus growth is impaired in cells that lack key components of the DNA damage response and are prone to accumulate point mutations [72,79]. Restriction on viral growth is explained by the fact that HSV exploits cellular response mechanisms to DNA damage for its own optimal growth, particularly for conducting uracil-initiated base excision repair [80,81,82]. However, we believe that HSV infection may induce accretion of point mutations in the host genome due to a delay of apoptosis. It is well known that HSV and other viruses prevent apoptotic cell death from proceeding which allows them to produce new progeny during infection cycle. As HSV penetrate into the nucleus, viral proteins, such as ICP 4, ICP 27 and US3 are instantly synthesized and involved in regulation of apoptosis, postponing cell dead [83,84,85].

3.6 Latent Infection and Genetic Instability

A latent infection is a phase of viral life cycle in which, after initial infection, virus production ceases. During a latent infection the viral genome is not fully eradicated and undergoes dramatic changes, forming episomes or insertions into the host genome. Infection of neurons by HSV, in particular those in the trigeminal and dorsal root ganglia, usually results in life-long latency of the virus in the host which is one of the most challenging features of HSV biology [86]. An HSV latent infection is characterized as episomal latency because viral DNA persists in the nucleus, in a circular episomal form, associated with nucleosomes. Other nuclear domains including promyelocytic leukemia nuclear bodies and centromeres are

functionally involved in the control of HSV-1 latency, and represent a key level of host/virus interaction [87].

While most of HSV genes are repressed in a latent infection, the latency-associated transcript (LAT) is intensively expressed. This results in several RNA species contributing to the establishment of latency. Recent findings showed that LAT not only regulates the establishment, maintenance and reactivation of virus from a latent state, but also inhibits the two major apoptotic pathways in neurons, caspase 8- and caspase 9-induced apoptosis [88-90]. LAT also inhibits the activation of caspase 3 that is implicated in apoptosis [91]. In LAT expressed neurons was detected a high level of protein kinase B (AKT), which serves as a key factor in many types of cancer [92]. Activation of AKT was shown to overcome cell cycle arrest in either phase G1 or G2 and may promote the survival and proliferation of cells [93,94]. Unblocked cells, due to ineffective checkpoint control may serve as an anchorage for mutations in DNA. In addition, Plo and Lopez reported that AKT represses homologous recombination induced by DSB, resulting in the accumulation of DSB in cells [95]. The correlation between LAT expression and high level of AKT needs detailed investigation in the near future as well as a link between LAT and genetic instability.

There is little evidence that the long-term persistence of HSV may directly have an influence on host genome stability, leading to the various types of rearrangements. As shown by Chenet-Monte and Heilbronn some viral proteins can be involved in the chromosomal aberrations and DNA amplification in cells during both forms of infection [96,97]. More recent results indicated that latent HSV infection in the murine nervous system is usually associated with focal chronic inflammation and with oxidative damage which can be expressed by the formation of chromosomal rearrangements [98,99,100].

4. CONCLUSION

As mentioned previously, cells have evolved mechanisms that are preventing them from various exogenous and endogenous sources of DNA damage. Although these mechanisms occur in all cells, the problem of genetic stability seems to be unsolved enigma. While some forms of instabilities contribute to the adaptation of cells on molecular level, others make harmful influence for cells and even for whole organisms, leading to the diseases such as cancer.

The studies of virus-induced genetic instability have provided critical insights into key mechanisms of mutagenesis. Some viruses play essential roles in the initiation as well as progression of genetic instability, leading to the formation and maintenance of transformed phenotype. During the past decades we accumulated evidences that confirm the leading role of some viruses in genetic instability and carcinogenesis. On the other hand, there are many other viruses that have always been with us and their role in genetic rearrangements remains unexplored and needs for further investigation. Herpes simplex virus is thought to be one of the best candidates due to its wide distribution among population and long term persistency. Analysis demonstrated that HSV infection induced a general increase of the level of mutations. This type of response is thought to be compatible with the specific behavior of the virus. The further studies promise many more discoveries that may help to uncover new biological effects of HSV.

ACKNOWLEDGMENTS

We apologize to those whose important contribution could not be appropriately discussed and cited due to space limitation. We are most grateful to Haig Eskandarian (Institut Pasteur, France) for critical reading.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Maizels N. Immunoglobulin gene diversification. Annu Rev Genet. 2005;39:23-46.
2. Baker DJ, Jeganathan KB, Malureanu L, Perez-Terzic C, Terzic A, van Deursen JM. Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. J Cell Biol. 2006;172(4):529–540.
3. Draviam VM, Xie S, Sorger PK. Chromosome segregation and genomic stability. Curr Opin Genet Dev. 2004;14(2):120-125.
4. Saetta AA, Stamatelli A, Karlou M, Michalopoulos NV, Patsouris E, Aroni K. Mutations of microsatellite instability target genes in sporadic basal cell carcinomas. Pathol Res Pract. 2007;203(12):849–855.
5. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res. 2008;18(1):85–98.
6. Baute J, Depicker A. Base excision repair and its role in maintaining genome stability. Crit Rev Biochem Mol Biol. 2008;43(4):239–276.
7. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T. DNA Repair and Mutagenesis. American Society For Microbiology Press, Washington DC; 2006
8. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet. 1964;1(7335):702-3.
9. Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigenassociated hepatitis. Lancet. 1970;1(7649):695-8.
10. Durst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc Natl Acad Sci USA. 1983;80(12):3812-5.
11. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 1984;3(5):1151-1157.
12. zur Hausen H. Viruses in human cancers. Current Science. 2001;81(5):523–7.
13. Feitelson MA, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Lett. 2007;252(2):157-170.
14. Dandri M, Burda MR, Burkle A, Zuckerman DM, Will H, Rogler CE, Greten H, Petersen J. Increase in de novo HBV DNA integrations in response to oxidative DNA damage or inhibition of poly(ADP-ribosyl)ation. Hepatology. 2002;35(1):217-223.
15. Hino O, Kajino K, Umeda T, Arakawa Y. Understanding the hypercarcinogenic state in chronic hepatitis: a clue to the prevention of human hepatocellular carcinoma. J Gastroenterol. 2002;37(11):883-887.

16. Minami M, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, Okanoue T. Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. Oncogene. 2005;24(27):4340-8.
17. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev. 2000;14(23):3065-73.
18. Munger K, Howley PM. Human papillomavirus immortalization and transformation functions. Virus Res. 2002;89(2):213-218.
19. Varmus HE. The molecular genetics of cellular oncogenes. Annu Rev Genet. 1984;18:553–612.
20. Weinberg RA, Wigler M. Oncogenes and the molecular origins of cancer. Cold Spring Harbor Press; Cold Sprinh, NY; 1989.
21. Poffenberger KL, Roizman B. Studies on noninverting genome of a viable herpes simplex virus 1: presence of head-to-tail linkages in packaged genomes and requirements for circularization after infection. J Virol. 1985;53(2):587-595.
22. Quinlan MP, Chen LB, Knipe DM. The intranuclear location of a herpes simplex virus DNA-binding protein is determined by the status of viral DNA replication. Cell. 1984;36(4):857-868.
23. McNamee EE, Taylor TJ, Knipe DM. A dominant-negative herpesvirus protein inhibits intranuclear targeting of viral proteins: effects on DNA replication and late gene expression. J Virol 74. 2000;10122-10131.
24. Brodbeck WG, Anderson JM. Giant cell formation and function. Curr Opin Hematol. 2009;16(1):53-57.
25. Chen EH, Grote E, Mohler W, Vignery A. Cell-cell fusion. FEBS Lett. 2007;581(11):2181-2193.
26. Oftebro R, Wolf I. Mitosis of bi- and multinucleate HeLa cells. Exp Cell Res. 1967;48(1):39-52.
27. Deaven LL, Kreizinger JD. Cell fusion as a mechanism for the origin of eparin dy cells in vitro.Experientia. 1971;27(3):311-3.
28. Roizman B. Polykaryocytosis induced by viruses. PNAS. 1962;48:228-234.
29. Spear PG. Herpes simplex virus: receptors and ligands for cell entry. Cell Microbiol. 2004;6(5):401-410.
30. Atanasiu D, Whitbeck JC, Cairns TM, Reilly B, Cohen GH, Eisenberg RG. Bimolecular complementation reveals that glycoproteins gB and gH/gL of herpes simplex virus interact with each other during cell fusion. PNAS. 2007;104(47):18718-23.
31. Cocchi F, Menotti L, Dubreuil P, Lopez M, Campadelli-Fiume G. Cell-to-cell spread of wild-type herpes simplex virus type-1, but not of syncytial strains, is mediated by the immunoglobulin-like receptors that mediate virion entry, nectin1(PRR1/HveC/HigR) and nectin2 (PRR2/HveB). J Virol. 2000;74(8):3909–3917.
32. Campadelli-Fiume G, Amasio M, Avitabile E, Cerretani A, Forghieri C, Gianni G, Menotti L (2007) The multipartite system that mediates entry of herpes simplex virus into the cell. Rev Med Virol. 2007;17(5):313-326.
33. Subramanian RP, Geraghty RJ. Herpes simplex virus type 1 mediates fusion through a hemifusion intermediate by sequential activity of glycoproteins D, H, L, and B. Proc Natl Acad Sci USA. 2007;104(8):2903-8.
34. Tiwari V, Clement C, Duncan MB, Chen J, Liu J, Sukla D. A role for 3-O-sulfated eparin sulfate in cell fusion induced by herpes simplex virus type 1. Journal of General Virology. 2004;85(Pt4):805–809.

35. Tiwari V, ten Dam GB, Yue BY, van Kuppevelt TH, Shukla D. Role of 3-O-sulfated eparin sulfate in virus-induced polykaryocyte formation. FEBS Lett. 2007;581(23):4468-72.
36. Shieh MT, WuDunn D, Montgomery RI, Esko JD, Spear PG. Cell surface receptors for herpes simplex virus are eparin sulfate proteoglycans. J Cell Biol. 1992;116(5):1273–1281.
37. Shukla D, Liu J, Blaiklock P and 7 other authors. A novel role for 3-O-sulfated eparin sulfate in herpes simplex virus 1 entry. Cell. 1999;99(1):13–22.
38. Eggert US, Mitchison TJ, Field CM. Animal cytokinesis: from parts list to mechanisms. Annu Rev Biochem. 2006;75:543–566.
39. Brito DA, Rieder CL. Mitotic checkpoint slippage in humans occurs via cyc.lin B destruction in the presence of an active checkpoint. Curr Biol. 2006;16(12):1194–1200.
40. Duelli DM, Hearn S, Myers MP, Lazebnik Y. A primate virus generates transformed human cells by fusion. J Cell Biol. 2005;171(3):493–503.
41. Chenet-Monte C, Mohammad F, Celluzzi CM, Schaffer PA, Farber FE. Herpes simplex virus gene products involved in the induction of chromosomal aberrations. Virus Res. 1986;6(3):245-260.
42. Everett RD, Earnshaw WC, Findlay J, Lomonte P. Specific destruction of kinetochore protein CENP-C and disruption of cell division by herpes simplex virus immediate-early protein Vmw110. EMBO J. 1999;18(6):1526–1538.
43. Hobbs WE, DeLuca NA. Perturbation of cell cycle progression and cellular gene expression as a function of herpes simplex virus ICP0. J Virol. 1999;73(10):8245–55.
44. Liu M, Schmidt EE, Halford WP. ICP0 Dismantles Microtubule Networks in Herpes Simplex Virus-Infected Cells. PLoS One. 2010:5(6):e10975 doi: 10.1371.
45. Li H, Baskaran R, Krisky DM, Bein K, Grandi P, Cohen JB, Glorioso JC. Chk2 is required for HSV-1 ICP0-mediated G2/M arrest and enhancement of virus growth. Virology. 2008;375(1):13–23.
46. Love R, Wildy P. Cytochemical studies of the nucleoproteins of HeLa cells infected with herpes virus. J Cell Biol.1963;17(2):237–254.
47. Alliegro MA, Henry JJ, Alliegro MC. Rediscovery of the nucleolinus, a dynamic RNA-rich organelle associated with the nucleolus, spindle, and centrosomes. PNAS. 2010;107(31):13718-23.
48. Farnsworth A, Wisner TW, Webb M, Roller R, Cohen G, Eisenberg R, Johnson DC. Herpes simplex virus glycoproteins gB and gH function in fusion between the virion envelope and the outer nuclear membrane. PNAS. 2007;104(24):10187–92.
49. Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM. Chromosome Mutation II: Changes in Chromosome Number. An Introduction to Genetic Analysis (7th ed.). New York: W. H. Freeman; 2000.
50. Martinez CA, van Wely KHM. Are aneuploidy and chromosome breakage caused by a CINgle mechanism? Cell Cycle. 2010;9(12):2275-80.
51. Hagglund R, Roizman B. Role of ICP0 in the strategy of conquest of the host cell by herpes simplex virus 1. J Virol. 2004;78(5):2169–78.
52. Lomonte P, Sullivan KF, Everett RD. Degradation of nucleosome-associated centromeric histone H3-like protein CENP-A induced by herpes simplex virus type 1 protein ICP0. J Biol Chem. 2001;276(8):5829–35.
53. Lomonte P, Morency E. Centromeric protein CENP-B proteasomal degradation induced by the viral protein lCP0. FEBS Lett. 2007;581(4):658–662.
54. Storchova Z, Pellman D. From polyploidy to aneuploidy. Genome instability and cancer. Mol Cell Biol. 2004;5:45-54.

55. Fenwick ML, Walker MJ. Suppression of the synthesis of cellular macromolecules by herpes simplex virus. J Gen Virol. 1978;41(1):37-51.
56. Brandt CR, Galloway DA, McDougall JK. Synergistic interaction between HPV-18 sequences, herpes simplex virus infections and chemical carcinogens. Cancer Cells. 1987;5:179–186.
57. Matz B. Herpes Simplex Virus Causes Amplification of Recombinant Plasmids Containing Simian Virus 40 Sequences. J Gen Virol. 1989;70(Pt6):1347-1358.
58. Heilbronn R, zur Hausen H. A subset of herpes simplex virus replication genes induces DNA amplification within the host cell genome. Journal of Virology. 1989;63(9):3683–3692.
59. Lukonis CJ, Weller SK. Characterization of nuclear structures in cells infected with herpes simplex virus type 1 in the absence of viral DNA replication. J Virol. 1996;70(3):1751-1758.
60. Galloway DA, McDougall JK. Alterations in the cellular phenotype induced by herpes simplex viruses. J Med Virol. 1990;31(1):36-42.
61. Harnden DJ. Viruses, chromosomes and tumors. The interaction between viruses and chromosomes. In Chromosomes and Cancer, 152-180. Edited by J. German. New York: John Wiley & Sons; 2000.
62. Boiron M, Tanzer J, Thomas M, Hampe A. Early diffuse chromosome alterations in monkey kidney cells infected in vitro with herpes simplex virus. Nature. 1966;209(5024):737-8.
63. Aya T, Makino S, Yamada M. Chromosome Aberrations Induced in Cultured Human Leucocytes by Herpes Simplex Virus Infection. Proc Japan Acad. 1967;3:239-244.
64. Sahnazarov N. Cytogenetic changes associated with cellular infection with herpes simplex virus. Stud Cercet Virusol. 1973;24(6);477-89.
65. Hampar B, Ellison SA. Cellular alterations in the MCH line of Chinese hamster cells following infection with herpes simplex virus. PNAS. 1963;49:474-480.
66. Waubke R, zur Hausen H, Henle W. Chromosomal and Autoradiographic Studies of Cells Infected with Herpes Simplex Virus. J Virol. 1968;10:1047-54.
67. Hwang CBC, Shillitoe EJ. Analysis of complex mutations induced in cells by herpes simplex virus type-1. Virology. 1991;181(2):620-9.
68. Stanley P. Chromosome Damage Induced by Herpes Simplex Virus Type 1 in Early Infection. J Gen Virol. 1986;67(Pt10):2273-7.
69. Cuchet D, Ferrera R, Lomonte P, Epstein AL. Characterization of antiproliferative and cytotoxic properties of the HSV-1 immediate-early ICPo protein. J Gene Med. 2005;7(9):1187-99.
70. Morgan WF, Corcoran J, Hartmann A, Kaplan MI, Limoli CL, Ponnaiya B. DNA double-strand breaks, chromosomal rearrangements, and genomic instability. Mutat Res. 1998;404(1-2):125–128.
71. Varga T, Aplan PD. Chromosomal aberrations induced by double strand DNA breaks. DNA Repair. 2005;4(9):1038–1046.
72. Lilley CE, Carson CT, Muotri AR, Gage FH, Weitzman MD. DNA repair proteins affect the lifecycle of herpes simplex virus 1. PNAS. 2005:102(16):5844–5849.
73. Gregory DA, Bachenheime SL. Characterization of Mre11 Loss Following HSV-1 Infection. Virology. 2008;373(1):124–136.
74. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature. 2001;412(6842):95-99.
75. Huang K, Zemelman BV, Lehman R. Endonuclease G, a Candidate Human Enzyme for the Initiation of Genomic Inversion in Herpes Simplex Type 1 Virus. J Biol Chem. 2002;277(23):21071-21079.

76. Schlehofer JR, zur Hausen H. Induction of mutations within the host cell genome by partially inactivated herpes simplex virus type 1. Virology. 1982;122(2):471–475.
77. Pilon L, Royal A, Langelier Y. Increased Mutation Frequency after Herpes Simplex Virus Type 2 Infection in Non-permissive XC cells. J Gen Virol. 1985;66(Pt2):259-65.
78. Pilon L, Langelier Y, Royal A. Herpes Simplex Virus Type 2 Mutagenesis: Characterization of Mutants Induced at the hprt Locus of Nonpermissive XC Cells. Mol and Cellular Biol. 1986;6(8):2977-83.
79. Muylaert I, Elias P. Contributions of nucleotide excision repair, DNA polymerase eta, and homologous recombination to replication of UV-irradiated herpes simplex virus type 1. J Biol Chem. 2010;285(18):13761-8.
80. Lilley CE, Schwartz RA, Weitzman MD. Using or abusing: viruses and the cellular DNA damage response. Trends Microbiol. 2007;15(3):119–26.
81. Bogani F, Chua CN, Boehmer PE. Reconstitution of Uracil DNA Glycosylase-initiated Base Excision Repair in Herpes Simplex Virus-1. J Biol Chem. 2009;284(25):16784–90.
82. Bogani F, Corredeira I, Fernandez V, Sattler U, Rutvisuttinunt W, Defais M, Boehmer PE. Association between the herpes simplex virus-1 DNA polymerase and uracil DNA glycosylase. J Biol Chem. 2010;285(36):27664-72.
83. Galvan V, Roizman B. Herpes simplex virus 1 induces and blocks apoptosis at multiple steps during infection and protects cells from exogenous inducers in a cell-type-dependent manner. PNAS. 1998;95(7):3931–3936.
84. Aubert M & Blaho JA. Modulation of apoptosis during herpes simplex virus infection in human cells. Microbes Infect. 2001;3(10):859-66.
85. Nguyen ML, Blaho JA. Apoptosis during herpes simplex virus infection. Adv Virus Res. 2007;69:67-97.
86. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer P, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla1 TA. Mitochondrial DNA Mutations, Oxidative Stress, and Apoptosis in Mammalian Aging. Science. 2005;309(5773):481-4.
87. Roizman B & Sears AE. An inquiry into the mechanisms of herpes simplex virus latency. Annu. Rev. Microbiol. 1987;41:543-571.
88. Catez F, Picard C, Held K, Gross S, Rousseau A, Theil D, Sawtell N, Labetoulle M, Lomonte P. HSV-1 genome subnuclear positioning and associations with host-cell PML-NBs and centromeres regulate LAT locus transcription during latency in neurons. PLoS Pathog. 2012;8(8):doi: 10.1371
89. Henderson G, Peng W, Jin L, Perng GC, Nesburn AB, Wechsler SL, Jones C. Regulation of caspase 8- and caspase 9-induced apoptosis by the herpes simplex virus type 1 lantency-associated transcript. Journal of NeuroVirology. 2002;8(2):103–111.
90. Jin L, Peng W, Perng GC, Brick DJ, Nesburn AB, Jones C, Wechsler SL. Identification of herpes simplex virus type 1 latency-associated transcript sequences that both inhibit apoptosis and enhance the spontaneous reactivation phenotype. Journal of Virology. 2003;77(11):6556–61.
91. Kent JR, Kang W, Miller CG, Freser NW. Herpes simplex virus latency-associated transcript gene function. Journal of NeuroVirology. 2003;9(3):285–290.
92. Carpenter D, Hsiang C, Brown DJ, Jin L, Osorio N, BenMohamed L, Jones C, Wechsler SL. Stable cell lines expressing high levels of the herpes simplex virus type 1 LAT are refractory to caspase 3 activation and DNA laddering following cold shock induced apoptosis. Virology. 2007;369(1):12–18.

93. Li S, Carpenter D, Hsiang C, Wechsler SL, Jones C. Herpes simplex virus type 1 latency-associated transcript inhibits apoptosis and promotes neurite sprouting in neuroblastoma cells following serum starvation by maintaining protein kinase B (AKT) levels. J Gen Virol. 2010;91(Pt4):858–866.
94. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, Roberts TM, Sellers WR. Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. PNAS. 1999;96(5):2110–5.
95. Kandel ES, Skeen J, Majewski N, Di Cristofano A, Pandolfi PP, Feliciano CS, Gartel A, Hay N. Activation of Akt/protein kinase B overcomes a G(2)/m cell cycle checkpoint induced by DNA damage. Mol Cell Biol. 2002;22(22):7831–41.
96. Plo I, Lopez B. AKT1 represses gene conversion induced by different genotoxic stresses and induces supernumerary centrosomes and aneuploidy in hamster ovary cells. Oncogene. 2009;28(22):2231-7.
97. Chenet-Monte C, Mohammad F, Celluzzi CM, Schaffer PA, Farber FE. Herpes simplex virus gene products involved in the induction of chromosomal aberrations. Virus Res. 1986;6(3):245-260.
98. Heilbronn R, zur Hausen H. A subset of herpes simplex virus replication genes induces DNA amplification within the host cell genome. Journal of Virology. 1989;63(9):3683–92.
99. Valyi-Nagy T, Olson SJ, Valyi-Nagy K, Montine TJ, Dermody TS. Herpes simplex virus type 1 latency in the murine nervous system is associated with oxidative damage to neurons. Virology. 2000;278(2):309-321.
100. Ventura I, Russo MT, De Luca G, Bignami M. Oxidized purine nucleotides, genome instability and neurodegeneration. Mutat Res. 2010;703(1):59-65.

© 2013 Zakaryan and Karalyan; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=200&id=8&aid=1151