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VIRUSI DANAS I SUTRA - ONKOGENI POTENCIJAL VIRUSA

VIRUSES TODAY AND TOMORROW - ONCOGENIC POTENTIAL OF VIRUSES

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Apstrakt

Savremena znanja pokazuju da virusna infekcija ne izaziva samo akutnu bolest, nego i perzistentne virusne infekcije, kao i da izvestan broj virusa može da inkorporira svoju nukleinsku kiselinu u genom ćelije domaćina, da dovede do rearanžiranja ćelijskih gena, te do pojave malignih tumora. Virusna perzistencija može da bude uzrok manifestacija latentne ili hronične infekcije, kao i prionima uzrokovanih sporih infekcija centralnog nervnog sistema. U nastanku stanja virusne perzistencije važnu ulogu imaju defektne Di virusne partikule. Virusi igraju značajnu ulogu u raznim poremećajima imunološke homeostaze organizma..

INTRODUCTION

We are witnessing a great increase of viral diseases in the world, some of them detected in the last decades. Some of these diseases such as AIDS and SARS, have permanently distinguished our century [1,2]. Over 60% of all infectious diseases are caused by viruses and this number is constantly increasing [1,3]. The awareness of the important role viruses play in the immune homeostasis disorder has changed some earlier laws in immunology and virology [4,5].

Techniques of molecular biology, immunology, enzymology and genetic engineering have been developed, as well as new knowledge on viruses and new standpoints in regard to development of virus diseases, their prevention, diagnosis and treatment. However, virus diseases being exclusively the topic in the frame of virology were only the ”tip of the iceberg”.

Current knowledge of viral infections indicates that they cause not only acute diseases (in acute cases, viral particles disappear from the host cell after remission). It is known nowadays that viruses can cause so-called persistent infections and remain within the cells even following the acute course of the disease [6,7,8].

Viral persistence in cells has several aspects. It may appear in the form of long-term chronic infections. In such infections, the virus continually replicates in spite of the immune reaction of the organism [2,6,7,8].

Other forms of viral persistence are latent infections. In such infections the virus remains in the host organism in a latent form and with decrease of immunity, replication of latent viruses starts [9,10, 11].

Special forms of long-term viral persistence are slow infections. Slow infections always and only involve the central nervous system. Considering the fact that there is no immune reaction or it is very weak, causes of these progressive encephalopathies in animals and humans are examined and thus prions are detected [12,13,14].

Prions consist only of a specific kind of protein. Nucleic acid has not been established in them, so virologists call them defective viruses (contrary to opinions of molecular biologists) [1,2,3,15,16,17].

Some defective viruses, such as hepatitis D virus, have been studied thoroughly. However, during long-term replication of each virus population within the host cells, a certain number of defective viruses are created [16]. These defective - Di particles can protect the cell from cytolysis. Defective viruses are considered to have, among other things, an important role in development of viral persistence [2,3,16].

Viral persistence indicates a wide spectrum of interactions: effects of host cell on the virus, which is one of the causes of viral variability, and effects of virus on the cell, which causes its transformation and even malignant alterations [2,18,19,20]. Viral persistence within the cell is the very reason why current virology was excluded from the frame of microbiology in the world, and that is why virology is being infiltrated into almost all branches of medicine at present, from immunology and genetics to oncology.

Disorder of immune homeostasis caused by viruses may develop due to mechanism of complete or abortive viral replication in lymphocytes and macrophages; effects of soluble cytokine factors; effects on antigen presenting cells, on cells involved in the process of phagocytosis, and excessive stimulation of the suppressor function of T-lymphocytes. The immune system is sometimes affected by several mechanisms [31].

The most interesting discovery in virology is the fact that viruses can incorporate their nucleic acid into the cell genome (into the cell DNA). In such a form, as pro-viruses, they remain in the cell for a longer period of time showing no visible activities. The cell multiplication is normal, whereas daughter cells and granddaughter cells contain proviral DNA incorporated in their genome. This may lead to rearrangements in the cell genome and to various changes -

malignant cell transformation being the most severe - causing development of virus-induced malignant tumors [21, 22].

Cancer is a complex molecular disease caused by a great number of factors. Furthermore, apart from cancerogenic factors, including viruses, immune system plays an important role (breakdown of immune control mechanisms, for whatever reason) [2, 23].

Virus-associated transformations induce alterations within the host cells, thus changing their phenotypic characteristics. Loss of contact inhibition occurs during multiplication of such cells, causing invasive and infiltrative tumor spread, metastasizing later on due to spreading by blood and lymph. Changed DNA index (due to aneuploidia) regarding to normal cells (diploid) can be established by flow cytometry. At the same time, new tumor antigens are formed within transformed cells. Virus-induced tumor antigens are, at least at the beginning, good transplantation antigens. (If a tumor is transplanted to a laboratory animal at the beginning of its growth as an allogenic transplant, cytotoxic T-lymphocytes specific for haplotype are formed) [1,2,23].

The immune system of the organism can recognize such tumor antigens and reject the transformed cells using the mechanism of immune control (usually contact destruction, using cytotoxic T-lymphocytes, unless the target tumor cell is coated with facilitatory antibodies). Immune control mechanisms prevent a certain number of occasional, initial and individual cell transformations in the organism. However, this property of tumor antigens quickly disappears and these altered cells further get out of control of immune control mechanisms [2, 23].

Analyses including nucleic acids of healthy and malignant cells and viral nucleic acids, using current techniques (Polymerase Chain Reaction - PCR in combination with hybridization methods) enabled genome mapping and detailed investigation of various genes. Analyzing certain regions of nucleic acids and their functions (coding, regulatory and supervisory in regard to gene repression and depression), cell and viral oncogenes have been detected. A certain number of authors consider cell oncogenes (which are in the phase of repression in normal cells) as viruses incorporated into cell DNA during the process of evolution [23,24].

Viral oncogenes of some viruses are already well known. They have their precise localization in the map of virus genome, as well as specific names. For example, in the family of Retroviridae, the only RNA viruses with oncogenic potentials, three characteristic gene sequences have been established: ”gag”, ”pol” and ”env”. ”Pol” is short for polymerase, the enzyme class reverse transcriptase which converts viral RNA into a single-stranded complementary DNA.

Double-stranded DNA formed by additional replication is incorporated into the cell genome as a circulating pro-viral DNA. By cell replication, pro-viral DNA is transferred to daughter cells. ”Gag” and ”env” sequences are viral proteins, which also have a role in oncogenesis. Analyzing genomes of oncoviruses from the family of Retroviridae incorporated into the genome of transformed cells, it has been established that all these viruses have lost replication genes. Their place is occupied by a sequence of virus nucleic acid called viral oncogene, which has malignant potentials. In order to provoke malignant change, oncogene expression is necessary [24,25].

Apart from viruses from the Retroviridae family (the only RNA viruses with oncogenic potential) in regard to DNA viruses, representatives of the following families have oncogenic potential: Papillomaviridae (Human papillomavirus - HPV), Herpesviridae, Adenoviridae (only in animals - hamsters), Hepadnaviridae (Hepatitis-B virus) and some animal viruses from the Poxviridae family. Oncogenic potential has not been established only in the DNA of Parvoviridae family (DNA virus family with single stranded DNA). The oncogenic potential of so many families of DNA viruses (in regard to only one family of RNA viruses) is understandable, because DNA viruses do not need a special enzyme (such as reverse transcriptase) to incorporate their DNA genome into the cell DNA.

Genetic manipulations in DNA viruses with oncogenic potential revealed that incorporation of viruses into cell genome in some cases leads to fusion with cell genes consequently causing structural and functional alterations. Affected by viruses, cellular oncogenes are converted into active oncogenes. After integration and fusion of viral DNA with cell DNA, virus replication stops. The only viral genetic information transcribed is the one responsible for tumor antigen synthesis and cell transformation [26,27].

In order to follow up aberrations of oncogene expression, as well as the role and importance of oncoproteins in all those occurrences, cell cultures are used, most often continuous lines of cervix carcinoma (CaSki, SiHa, Hela). Aiming to analyze presence of viruses and viral genome in cells, hybridization techniques are used (Southern blot for DNA and Northern blot for RNA viruses) as well as PCR methods [26,27,28].

In analysis of oncogenic potential of human papillomaviruses (HPV), gene expression of these viruses is applied. Prokaryotic and eukaryotic systems are used as expression vectors as well as their expression mean values. (pEx vectors are usually used as prokaryotic expression vectors, typical for temperature-sensitive strains of E. coli, whereas in regard to eukaryotic expression vectors - Bucalovirus is used, one of the most recent achievements of molecular biology in this field - expression vector typical for insect cells) [28].

It is not easy to interpret data obtained in investigations of viral oncogenic potential due to many reasons. Viruses cannot always be detected by available methods. That is why such investigations used to be long-lasting and often without results. Introduction of new, current methods for detecting viral nucleic acids, even if they are incorporated in the cell genome, has provided better results and new data on viruses and cancerogenesis [2,24,29]. PCR and hybridization techniques provide not only reliable detection of viral genome in transformed cells, but also detection of viral nucleic acid sequence and their detailed investigation.

HPV causes various benign and malignant skin and mucosal lesions (mouth, larynx, anogenital region). The following HPV genotypes are associated with oncogenesis: 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 57, 58 and 59 [24,27Ć. Zur Hauzen won the Nobel Prize in 2008 for his work on human papilloma viruses (the description of the natural history of HPV infection, an understanding of how HPV-induced cancerous cells are produced and the development of vaccines).

MATERIAL AND METHODS

With applied hybridization technique in situ, we detected certain HPV genotypes using 80 preparations taken from cervix uteri of women from our environment. Histologic changes occurred in cells in which certain HPV genotypes were found as well.

Tissue samples taken from the site of lesion - uterine cervix - were formalin-fixed parafin-embedded and hematoxylin-eosin stained for histopathologic examinations and then used for in situ hybridization in the aim of detecting the following HPV genotypes: 16, 18, 31, 33, 35 (associated with malignant transformations) and genotypes 6 and 11 which are not considered to be risky genotypes. In order to estimate wether it was an intraepithelial neoplasm (or not) the following criteria are used: typical histologic and cytologic changes associated with aneuploidy in the surface epithelium. In all biopsy samples chosen for assessment of virus DNA HPV in situ (in cells taken from uterine cervix) koilozytosis was diagnosed (histopathologic indices of viral infection).

In situ hybridization used for detection of DNA sequences of certain HPV genotypes was performed using biotin-labeled HPV-DNA probes. Readings were based on colorimetric in situ hybridization technique. Detection of the biotin-labeled DNA probe complex and the complementary HPV genome sequence was visualized using conjugates of alcaline phosphatase, which specifically binds to probes.

Dephosphorilization of 5-bromo-4-chloro-3-indoyl-phosphate substances stained with nitroblue tetrazolium, resulted in violet precipitation at the site of probe binding. It meant a positive result of viral DNA and certain HPV types in cells (in situ). The test included three special groups of probes for detecting HPV genotypes: HPV 6 and 11, HPV 16 and 18, as well as HPV 31, 33 and 35, then positive DNA probe-specific for human sequence of DNA genome and negative DNA probe-specific for plasmid vector (biotin-labelled vector pBR322 DNA).

RESULTS AND CONCLUSION

Table 1 presents our results in detecting certain HPV genotypes by hybridization technique in situ, using 80 preparations taken from cervix uteri of women from our environment. Histologic changes occurring in cells in which certain HPV genotypes were detected are presented as well.

Table 1 and graph show that HPV genotypes 16, 18, 31, 33, 35, 6 and 11 are detected in 33.33% of samples taken from cervix uteri samples of women from our surroundings. Genotypes 6 and 11 (not associated with malignant transformations) were detected in 8.33% of samples. The incidence of HPV 16 and 18 genotypes was the highest (21.67% of cases), whereas the incidence of 31, 33 and 35 genotypes was the lowest - only 3.33% of samples.

Our results confirm the efficacy and sensitivity of DNA HPV in situ hybridization and confirm that certain HPV genotypes are found in genital lesions of women from our environment. It is necessary to continue such studies in order to assess the correlation of HPV types with certain lesions of the genital tract diagnosed as benign changes, precancerous conditions and malignant lesions. This would facilitate creation of programs of prevention, early detection and treatment of malignant tumors.

We hope that mankind will succeed in solving the problem of controlling cancer and that we shall celebrate victory on this complex path for medicine and biology. All, even the smallest investigations in this field, represent a contribution to a permanent fight of mankind to acquire new knowledge, win new victories and fight for life itself.

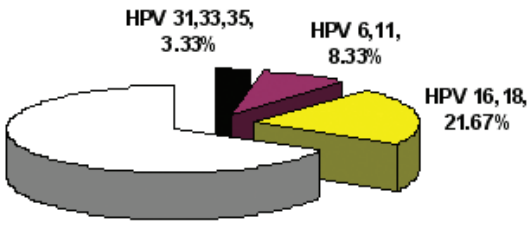
Table 1. Results of detection of certain Human Papillomavirus (HPV) genotypes by in situ hybridization and histopathologic alterations evident in cells (lesion site - uterine cervix)

Tabela 1. Rezultati detekcije određenih genotipova humanih papiloma virusa (HPV) tehnikom hibridizacije in situ i patohistološke promene uočene u ćelijama (mesto lezije cervix uteri)

Broj ispitanih Number of examinees	+hibridizacija/ + hybridization	Genotip HPV HPV genotype	KERATOZA/KERATOSIS				
			K	AM	P	D	H
10	2	16,18	+	+	+	-	+
9	2	31,33,35	+	+	+	+	+
	2	16,18					
6	3	16,18	+	+	-	+	-
11	4	16,18	+	+	-	-	+
8	3	6,11	+	-	+	-	-
4	2	6,11	+	-	-	-	-
10	2	16,18	+	-	-	-	+
2	0	0	+ /-	-	-	-	+ /-

Legenda:
K - koilociti;
AM - atipične mitoze;
P - parakeratoza;
D - displazija;
H - hiperplazija;
+/- - sasvim početne promene

Legend:
K - koilocytes;
AM - atypical mitoses;
P - parakeratosis;
D - displasia;
H - hyperplasia;
+/- - initial changes



Graph. Incidence of certain genotypes of human papilloma virus (HPV) in lesions of uterine cervix

Grafikon. Učestalost pojedinih genotipova humanih papiloma virusa (HPV) u lezijama grlića materice

Abstract

The current studies show that viral infections can cause not only acute, but also persistent viral diseases. A certain number of viruses are able to incorporate their nucleic acid into the genome of the host-cell, leading to rearrangement of the cell genes and formation of malignant tumors. Viral persistence can cause manifestations of latent or chronic infections, as well as prion-caused slow infections of the central nervous system. Defective Di particles play an important role in maintaining viral persistence. Viruses are important agents involved in various disorders of the immune homeostasis of the organism.

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