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Oncology

VIRAL CARCINOGENESIS: A REVIEW

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ABSTRACT Certain DNA and RNA viruses that are associated with Tumors have supported cancer research in two major aspects firstly, to discover and access the control pathways of cellular growth along with formulating the current concepts of cancer biology and, second, to discuss the etiology of some cancers occurring in Humans. In many Transforming retroviruses growth control and mitogenic signalling are found to be associated with oncogenes derived from cellular genes. The discovery of tumor suppressors is corresponding to these oncogenes of viral origin that the DNA tumor viruses encode which are found to be essential for viral replication and Host cell transformation. These virus-host complexes stimulate cell cycle progression. This line of thought states that cancer develops by the accumulation of multiple cooperating events. Viruses including hepatitis B & C virus, Epstein–Barr virus, human T-cell leukemia virus type I and Human papilloma viruses are now considered as genuine causative factors of human cancer. It is the infectious nature of viruses resulting in chronic long-standing infections in humans that acts as a distinguishing factor between them from all other cancer etiologies; with the development of cancer being an unfortunate aftereffect of viral replication. Human Viruses usually do not act as definite carcinogens, and these viruses have different roles in cellular transformations further leading to carcinogenesis. It may take years between primary infection and tumorigenesis and in a number of infected cases cancer development might not even occur, although viral carcinogenesis is predominantly seen in immunocompromised individuals. This article is an effort to explain the Theories and mechanism of viral carcinogenesis including the Factors contributing to viral carcinogenesis such as the Viruses associated, viral oncogenes and deregulation of host cell function. The article will also try to put emphasis on Diagnosis of viral induced carcinogenesis.

KEYWORDS :

INTRODUCTION

Viruses have been key instruments in the revolution of cancer biology over the last 20 years. Without viral carcinogenesis, it is difficult to conceive that the molecular basis of cancer would stand revealed so clearly today. Although viewed originally as unusual agents that caused cancer in animals and were thought to be of no particular relevance to humans, viruses have turned out to be the 'Rosetta stone' for unlocking the mysteries of cell growth control.[1]

HISTORICAL BACKGROUND

The tumor viruses have played two major roles in cancer research over the last 2 decades: First, as tools for the discovery and dissection of cell signaling and growth control pathways and, second, as newly appreciated causative agents of human neoplasia.[1]

The first evidence of tumor viral aetiology dates back to 1907 when Ciuffo and co-workers showed that human warts could be transmitted by cell-free filtrates derived from lesions.[2] Seventy years later, papilloma viruses were linked to human cancer.[2] In 1908, Ellermann and Bang, reported that also leukemia could be transferred to healthy chicken by a cell-free filtrate of cells obtained from affected birds.[2] Moreover, in 1911, Rous and colleagues showed that the spindle cell sarcoma could be transmitted to healthy chickens using filtered cell-free tumor extracts. This study led to the identification of the first oncogenic virus: 'the Rous sarcoma virus' (RSV).[2] As far as primates are concerned, in 1962, Eddy, Hilleman, and co-workers showed the tumorigenic potential of Simian Virus 40 (SV40) while, Trentin and colleagues reported, for the first time, that viruses could be linked to cancer development also in humans, under experimental conditions.[2] In 1965, Epstein, Barr et al were able to visualize by electron microscopy, a herpes virus-like particle in a cell line, established from Burkitt's lymphoma (BL).[2] It is now estimated that 20%-25% of human cancers worldwide have a known viral etiology.[3] Viruses linked to carcinogenesis include several DNA viruses such as Kaposi's sarcoma herpes virus (KSHV), Merkel cell polyoma virus (MCV), Epstein-Barr virus (EBV), Human papilloma virus (HPV), hepatitis B virus (HBV) and the simian virus 40 (SV40), as well as RNA viruses such as human T-lymphotropic virus-1 (HTLV-1) and the hepatitis C virus (HCV).[4] Viral proteins may directly act as oncogenes that drive cells to proliferate or generate inflammatory responses and cause regeneration of injured cells that eventually lead to malignant transformation.[4] Accelerated viral carcinogenesis is observed in the immune-deficient host.[5] Decreased T-cell reactivity and lower number of antigen-presenting cells in the skin assist in viral escape and emergence of skin tumors.[6]

THEORIES OF CARCINOGENESIS

TABLE NO. 1: Five recent non-exclusive theories of carcinogenesis: 10

THEORY	Genome instability	Non-genotoxic or epigenetic	Mutational	Tissue organization	Darwinian
Main focus	Familiality Genome instability	Clonal expansion/epigenetics	Chemical carcinogens	Microenvironment Morphostats	Clonal expansion/cell selection
Examples	Colon cancer Rb	Diet, hormones	Viruses Tobacco and lung HPV		Beta-carotene, folate, chemotherapy
Mechanisms	CIN, MIN, MMR, Rb, BRCA1, TSG	Methylation histone Acetylation	DNA adducts Mutations Oncogenes		Selective advantage

1) Aneuploidy or genome instability theory:

Recently the 'aneuploidy hypothesis', first proposed by Theodor Boveri 1914, is gaining momentum.[9] According to this hypothesis, a carcinogen through a preneoplastic aneuploidy, which destabilizes mitosis, initiates carcinogenesis.[9] This in turn initiates an autocatalytic karyotype evolution that leads to the generation of new chromosomal variants, including rare neoplastic aneuploidy (Duesberg et al. 2001).[9] It is characterized by two, almost parallel, lines of research. First was the theory developed by Knudson for retinoblastoma, which became the basis of the 'two-hit' hypothesis (1971) and led to the formulation of the theory of 'tumor suppressor genes' (TSGs) and then to the discovery of Rb1 (Friend et al., 1986).[9] In the second line of research, the

investigation of familial colon cancer led to the discovery of 'microsatellite instability' (MIN) and 'mismatch repair' genes.[9] The basic thought is that changes in some genes that regulate chromosome stability or repair of DNA damage lead to a cascade of events and grossly increase the frequency of mutations downstream.[9] The 'two-hit model' originally proposed by Knudson and later confirmed by the discovery of the Rb gene, implicates the impact of mutations in recessive TSGs (both alleles need to be mutated), which is dissimilar to the role of mutations in dominant oncogenes (one mutation is sufficient).[9] In the first model, the gene controls genome stability and in the second model, the gene directly encodes for a gene product which controls cell proliferation or other neoplastic phenotypes.[9]

2) Epigenetic theory or non-genotoxic theory:

Nowell (1976) emphasized chromosomal instabilities. [7] It has been conceded that non-mutational stable changes take place in cellular genomes, which lead to carcinogenesis (Feinberg 1993 Cross and Bird 1995). [7] Such events involve DNA methylation, genome imprinting and changes in DNA – nucleoprotein structure and are termed 'epigenetic'. [7] Spontaneous mutation rates in the affected human gemone are a result of increased levels of methylated cytosine (one of the pyrimidine bases in DNA) (Balmain 1995).[7] This theory is more recent and lays emphasis on non-genotoxic effects. It states that several important modulators of cancer risk such as diet, obesity, hormones and insulin resistance, do not act through a structural change in DNA but rather through functional changes including epigenetic events.[9]

3) Gene mutation theory or Somatic mutation theory:

Whitman (1915) introduced the notion that the cancer cell was a "mutated" cell, and coined the idea of a somatic mutation to explain what Boveri had implied in his 1914 narrative.[9] This theory states that somatic gene mutations form the basis of neoplastic transformation and their clonal expansion, which lead to carcinogenesis. [9] This theory is the most widely accepted theory and is supported by a lot of experimental data (review by Bishop 1987).[9] However, it does not describe the tumor heterogeneity, aneuploidy and also, the long latent periods between exposure to carcinogens and the cancer development.[7] It is the field of interest in cancer research for the last 50 years. It is based on the following attributes: 1) Cancer is derived from a single somatic cell that successively has accumulated multiple DNA mutations (monoclonality), 2) Those mutations occur on genes that control cell proliferation and cell cycle and 3) The default state of cell proliferation in metazoa is *quiescence*.[8] This theory assumes that organismic phenomena can be conveniently reduced to cellular and/or subcellular levels.[9] To oblige the inconsistencies that might lead to the invalidation of the Somatic mutation theory, another alternative was proposed termed as *ad hoc* alternative, by which oncogenes would exercise their effects indirectly by affecting tissue organization, in addition to disrupting the proliferation control of the cells that harbor them.[8]

4) Tissue organization field theory:

In 1999, on the basis of the work on the control of cell proliferation and a comprehensive analysis of the literature, an alternative theory was proposed by C Sonnenschein and AM Soto which they termed as the "*tissue organization field theory"* (TOFT).[8] It considers that 1) carcinogenesis is a problem of tissue organization, comparable to organogenesis during early development, and 2) *proliferation* is the default state of all cells.[8]

The TOFT anticipates the neoplastic phenotypes to be potentially reversible through cell-cell and/ or tissue-tissue interactions.[8] This has been verified experimentally, example being the normalization of teratocarcinoma cells injected into blastocysts.[8] Moreover, nuclei from a variety of cancer cells transplanted into enucleated oocytes led to normal pre- and post-implantation development.[8]

5) Darwinian interpretation theory:

Thanks to the work of Greaves and, from a mathematical point of view, of Nowak; this interpretation—attributes a greater role to clonal expansion (selection) of cells rather than to mutations but puts emphasis on the role of the environment (both macro and micro) in selecting cells that have some acquired advantage. [9] In fact, the term Darwinian needs to be used cautiously, being a short cut for somatic cellular selection.[9] It has entered into use in the cancer literature, but it should not be used to imply that Darwinian selection at the population (rather than cellular) level is involved in carcinogenesis.[9]

VIRUSES ASSOCIATED WITH CARCINOGENESIS:

TABLE NO. 2: Human oncogenic viruses.[3]

Taxonomic Grouping	Examples	Oncogenes	Tumor Types
1. DNA viruses	Adenovirus	E1A, E1B	Various solid tumors
-Adenoviridae	type 12, 18,	HBx	only in rodents
-Hepadnaviridae	31	LMP-1,	Hepatocellular
-Herpesviridae	HBV	BARF-1	carcinoma
	EBV	VGPCR	Burkitt's lymphoma,
-Papovaviridae	KSHV	T antigen	B-cell lymphoma,
	Merkel cell		NPC
-	polyoma. V	E6, E7	Kaposi sarcoma,
Papillomaviridae	BK virus, JC		primary effusion
2. RNA Viruses	virus		lymphoma
-Flaviviridae	HPV	?	Merkel cell carcinoma
Hepacivirus	16,18,31,45		Solid tumor in rodents
-Retroviridae	Hepatitis C	Tax	and primates
HTLV	virus		Cervical and anal
	Human T-		cancer, Oral cancer
	cell leukemia		Hepatocellular
	virus type I		carcinoma
			Adult T-cell
			leukemia/lumphoma

Tumor viruses can be classified into two groups based on their genetic material, as summarized in Table 2. Cancer-causing DNA tumor viruses and RNA-containing retroviruses have been extensively investigated.[3]

1. Human DNA tumor viruses

Human Papilloma Viruses (HPVs)

Human papillomavirus (HPV) is a small, non-enveloped deoxyribonucleic acid (DNA) virus that infects skin or mucosal cells.[3] The circular, double-stranded viral genome is approximately 8-kb in length. The genome encodes for 6 early proteins responsible for virus replication and 2 late proteins, L1 and L2, which are the viral structural proteins.[3] High-risk or oncogenic HPVs are etiological agents of cervical cancer.[3] Among the high-risk HPVs, HPV16 and HPV18 are the principal causes of cervical cancer as well as several other tumor types.[3]

Epstein-Barr virus (EBV)

The virus is approximately 122 nm to 180 nm in diameter and is composed of a double helix of DNA.[3] The DNA is surrounded by a protein nucleocapsid. This nucleocapsid is surrounded by a tegument made of protein, which in turn is surrounded by an envelope containing both lipids and surface projections of glycoproteins that are essential to infection of the host cell. [3] EBV primarily causes infectious mononucleosis, but also contributes to the pathogenesis of four human tumors: African form of Burkitt's lymphoma, B-cell lymphomas in individuals with immunosuppression, nasopharyngeal carcinoma (NPC) and some kinds of Hodgkin disease.[3] EBV infects B lymphocytes, but does not replicate within the B cells; instead, it transforms them into lymphoblasts, which have an indefinite life span, rendering these cells immortal. EBV encodes a viral oncogene, LMP1 (latent membrane protein-1 or BNLF1).[3]

Kaposi sarcoma-associated herpes virus (KSHV)

KSHV is a large double-stranded DNA virus with a protein covering that packages its nucleic acids, called the capsid, which is then surrounded by an amorphous protein layer called the tegument, and finally enclosed in a lipid envelope derived in part from the cell membrane. [3] KSHV or human herpes virus 8 (HHV8) infection is associated with all forms of Kaposi sarcoma, primary effusion lymphoma or body cavity–based B-cell lymphoma, and multicentric Castleman disease.[3] KSHV encodes a viral G protein–coupled receptor (vGPCR) that presumably functions as a viral oncogene in immortalization of human endothelial cells and induction of angio-proliferative tumors.[3]

Human polyomaviruses

Polyomaviruses are DNA-based (double-stranded DNA) viruses.[3] They are small (40–50 nanometers in diameter), and icosahedral in shape, and do not have a lipoprotein envelope.[3] The most important member in this family is simian virus 40 (SV40), one of the most common latent viruses of rhesus monkeys.[3] Although two human polyomaviruses, BK virus and JC virus, have been described as oncogenic in rodents and nonhuman primates, whether these two viruses have any roles in human cancer is not clear.[3] Recently, a new human polyomavirus, Merkel cell polyomavirus (MCV), was discovered in ~80% of Merkel cell carcinomas (MCCs). An established MCC cell line contains monoclonal integrated MCV DNA.[3]

Human adenoviruses

Human adenoviruses are a group of small DNA viruses that commonly cause respiratory infections.[3] Adenoviruses (members of the family *Adenoviridae*) are medium-sized (90–100 nm), non-enveloped (without an outer lipid bilayer) viruses with an icosahedral nucleocapsid containing a double stranded DNA genome.[3] Human adenoviruses have not been linked to any human cancer, but some serotypes, such as adenovirus types 2, 5, 12, 18, and 31, are capable of trans-forming rodent cells in culture and inducing tumors in hamsters or rats.[3] Two viral oncogenes, E1A and E1B, have been identified as responsible for the adenovirus tumorigenicity and thus have served as useful tools for studying many important cellular processes in tumor biology.[3]

Hepatitis B virus (HBV)

Hepatitis B virus is a member of the Hepadnavirus family.[3] The virus particle, called Dane particle (virion), consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein.[3] The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells.[3] The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core.[3] HBV is endemic in Southeast Asia and sub-Saharan Africa.[3] Epidemiological observations and experimental evidence in animal models have established a clear association between HBV infection and liver cancer.[3] Although the precise role of HBV in causing liver cancer is not yet understood, some compelling evidence suggests that the HBx gene could be a viral oncogene, as its protein product can disrupt signal transduction and deregulates cell growth.[3]

2. Human RNA tumor viruses

Human T-cell leukemia/lymphoma virus (HTLV-1)

HTLV-1 is an enveloped virus that contains two identical copies of a plus single-stranded RNA genome and an outer envelope containing protruding viral glycoproteins.[3] HTLV-1–associated adult T-cell leukemia/lymphoma is endemic in the southern islands of Japan, the Caribbean basin, and South Africa.[3]

Xenotropic murine leukemia virus–related virus (XMRV)

Murine leukemia viruses produce a virion containing a spherical nucleocapsid (the viral genome in complex with viral proteins) surrounded by a lipid bilayer derived from the host cell membrane.[3] The lipid bilayer contains integrated host and viral proteins studded with carbohydrate molecules.[3] The viral particle is approximately 90 nanometres (nm) in diameter.[3] Recently, Dong et al in 2007 claimed that they discovered XMRV as a new human retrovirus associated with prostate cancer.[3] XMRV was isolated from prostate cancer tissue from patients homozygous for reduced enzyme activity of RNase L due to a single amino acid substitution and is susceptible to inhibition by interferon.[3]

Hepatitis C virus (HCV)

The hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin.[12] Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope.[12] While some HCV infections will have a spontaneous resolution, the majority will progress to chronic HCV.[12] The infection by HCV may be unresolved in approximately 85% of the infected individuals, representing an important cause of liver cirrhosis and hepatocellular carcinoma.[12] Although HCV oncogenesis is not well understood, persistent HCV infection is a prerequisite for the development of HCV-associated liver cancer.[3]

MECHANISM OF VIRAL CARCINOGENESIS:

DNA virus mechanisms underlying oncogenesis

To date, few DNA viruses are consistently associated with human neoplasms, notably the hepatitis B virus (HBV), human papilloma virus (HPV), EBV, and Kaposi sarcoma herpes virus (KSHV).[11] Cell immortalization is a pivotal phenomenon in carcinogenesis, also one of the major characteristics shared by malignant neoplasms.[11] In order to produce malignant tumors, cells must have to bypass apoptosis deflagrated during cell crisis, the endpoint of cellular senescence, observed when a critical level of telomere shortening is reached.[11] Moreover, major mechanisms that grant an increased lifespan to malignant cells are related to telomere maintenance and suppression of apoptosis, which will be discussed later.[11] Most human cancer cells have their telomeres lengthened by de novo expression of telomerase, an enzymatic complex formed by three major components: an RNA template of telomeric repeats (TTAGGG in vertebrates), the telomerase-associated protein (hTep-1), and a catalytic subunit that shows reverse transcriptional activity – the human telomerase reverse transcriptase (hTert).[11] Expression of TERT gene is rate limiting for telomerase activity, which is usually restricted to stem cells and germinal cells in non neoplastic tissues.[11] The RNA component is expressed constitutively and ubiquitously in normal cells.[11] Besides telomerase reactivation, telomeres may be subjected to the alternative lengthening of telomeres (ALT) pathway, a recombination-based mechanism for telomere lengthening.[11] Although ALT activation may not share the same frequency of telomerase de novo expression during cancer development, ALT has gained considerable attention due its anticipated role on cancer resistance to the new generation of drugs that target telomerase activity.[11]

TABLE NO.3: DNA viruses with well-documented association with human cancers.[13]

Virus	Viral taxonomy	Discovery	Genome	Associated human cancers
Hepatitis B virus	Hepadnaviridae	1967	dsDNA(partial) 3.2 kb, 4 genes	Hepatocellular carcinoma
Human Papilloma virus	Papillomaviridae	1983	dsDNA 8 kb	Squamous cell carcinomas in anogenital and head and neck sites
Epstein–Barr virus/ human herpes virus 4	Herpesviridae (gammaherpesvirus; lymphocryptovirus)	1964	dsDNA 172 kb, _90 genes	Burkitt lymphoma, undifferentiated nasopharyngeal carcinoma, Hodgkin lymphoma, non-Hodgkin lymphoma in immunosuppressed patients
Kaposi sarcoma associated Herpes virus/human Herpes virus 8	Herpesviridae (gammaherpesvirus; radnovirus)	1994	dsDNA 165kb, _90 genes	Kaposi sarcoma, primary effusion Lymphoma

Retroviral mechanisms underlying oncogenesis

There are various mechanisms by which oncogenic retroviruses induce malignancies.[10] Oncogenic retroviruses can be classified into two groups based on the mechanism underlying the disease: a) acute-transforming retroviruses and b) non-acute or slow-transforming or non-transforming viruses.[10] Acute-transforming retroviruses are typically replication defective viruses and rapidly induce tumors because of the viral oncogenes, 'proto-oncogenes'.[10] They form polyclonal tumors with a short latency after infection of the host, usually within two to three weeks.[10] This could be attributed to their high transformation capacity.[10] Transformation is mediated by the expression of viral oncogenes including *v-Abl* in the Abelson Murine leukemia virus (A-MuLV), which are virally encoded oncogenenic versions of normal cellular genes.[10]

Once captured by the virus, protooncogenes undergo mutations that lead to uncontrolled cell proliferation.[10] It is important to note that activation of cellular proto-oncogenes has been discovered in human cancers.[10] This can result from up-regulation of proto-oncogene

products by gene amplification or chromosomal translocation, or activation of proto-oncogene proteins by point mutations.[10]

Unlike acute-transforming retroviruses, non-acute or slow-transforming viruses are considered replication competent and do not carry oncogenes.[10] Tumorigenesis results from mutations caused by either promoter/enhancer insertion or by insertional mutagenesis.[10] During the promoter/enhancer insertion, non-acute transforming viruses can activate cellular proto-oncogenes by inserting a viral long terminal repeat (LTR) close to the proto-oncogenes to induce tumors.[10] Insertional mutagenesis is a common mechanism in rodent, feline, and avian retroviruses, where the retrovirus integrates into the host genome and affects the transcription of the neighboring genes. In general, non-acute-transforming retroviruses induce tumors with a prolonged latent period.[10]

TABLE NO. 4- DISCRIPTION OF RETROVIRUSES.[12]

Genera	Species
Alpharetroviruses	Avian sarcoma-leukosis virus, Rous sarcoma virus
Betaretroviruses	Mouse mammary tumor virus
Gammaretroviruses	Murine leukemia virus, Feline leukemia virus
Deltaretroviruses	Human T-lymphotrophic virus, Bovine leukemia virus
Epsilonretroviruses	Walleye dermal sarcoma virus
Lentiviruses	Human immunodeficiency viruses (HIV- 1 and HIV-2), Simian immunodeficiency virus, Feline immunodeficiency virus
Spumaviruses	Boviny foamy virus, Simian foamy virus

VIRAL ONCOGENES AND DEREGULATION OF HOST CELL FUNCTIONS

First and foremost, in order to establish itself in the host, it is indispensable for a virus to bring about uncoupling of cellular differentiation and proliferation, thus presenting its own genome an opportunity to replicate in the cycling infected cell. To attain this, oncogenic viruses have evolved a plethora of mechanisms to hijack different cellular processes described below:

Cell signalling

The most fundamental characteristic of a cell is to proliferate in a controlled manner in response to various growth signals and inhibitory stimuli. Tumor viruses through their oncoproteins and other regulatory molecules modulate nearly all major signaling pathways,[13] including MAP kinase, JAK-STAT, TGF, NF-B, Notch, TNF, Wnt and Hedgehog. It has been suggested that tumor viruses do so in order to create an ambience conducive for their replication and push host cells to actively divide and proliferate.[13]

Hedgehog (Hh) signalling is another key pathway reportedly activated in cancers of brain, skin and liver. HBx increases the stability and nuclear translocation of Gli-1, a key transcription factor of Hh signalling pathway. Blockade of Hh signalling impairs HBx ability to promote cell migration, anchorage-independent growth and tumour development.[13] Likewise, in chronic HCV infections, Hh ligands are upregulated and increased Hh signalling is associated with cirrhosis and HCC.[13] Hh-activating mutations are selected in cells immortalized by HPV. Inhibition of Hh pathway in cervical cancer cells renders them susceptible to apoptosis.[13]

Regulation of transcription

Viral oncoproteins usually reprogramme host cells by hijacking and repurposing host regulatory components of transcription network. Since a major requirement for induction of cell cycling is to overcome the Rb mediated repression of cell cycle-regulated genes, most tumor viruses deploy a vast repertoire of viral strategies to modulate Rb function.[13] These may include its hyperphosphorylation and thus inactivation, degradation and decrease in half-life of Rb, eventually leading to activation of E2F transcriptional activity.[13] Virus induced unscheduled inactivation of Rb triggers strong engagement of p53-mediated cell cycle arrest and cell death. Hence viruses have evolved elaborate mechanisms to circumvent p53-driven antiproliferative response.[13]

Regulation of replication and DNA damage

Replication of tumor viruses is intrinsically linked to their ability to drive cell proliferation. Most of these viruses infect quiescent cells driving their re-entry into cell cycle to promote an environment conducive for viral genome replication.[13] Such aberrant induction of cell proliferation results in replicative stress and elicits a DNA damage response (DDR).[13] Replication factors like PCNA, Cdt1, CDC6 and geminin have been found to be dysregulated by viral oncoproteins like E7 and HBx, which have been correlated with induction of re-replication.[13] Hence oncogenic viruses have developed mechanisms to directly activate specific components of DDR, while stringently inhibiting downstream triggering of cell death.[13] ATM(Ataxia telangiectasia mutated) arm of DDR pathway is frequently activated following HBV, KSHV, MCV and EBV infection.[13] Additionally, DDR pathway can also be activated indirectly through induction of mitotic defects HTLV-1, KSHV and HPV oncoproteins[13], and elevation of reactive-oxygen species by oncoproteins like Tax and EBNA1.[13]

Epigenetic reprogramming of tumor virus-infected cell

Orderly progression of DNA transcription, replication, recombination and repair requires spatial and temporal changes in the structure of the chromatin, which in turn governs the availability of gene regulatory elements, controlling their tissue-specific expression.[13] It is increasingly becoming clear that viral oncoproteins promote wide-spread remodeling of chromatin organization, contributing to both up- and down-regulation of a large number of genes.[13] Consistent with this concept, most viral oncoproteins exhibit interaction, enzyme activity stimulation and/or transcriptional up-regulation of DNMTs. In conclusion, virus-encoded oncoproteins hijack host epigenetic machinery to promote viral replication and expression of viral genes, in the process altering epigenetic signature of the host cell and triggering oncogenesis.[13]

Translational machinery

Viruses for their own protein synthesis and perpetuation often hijack cellular protein translational machinery which strongly correlates with cellular metabolic activities.[13] Different steps of translational machinery are reported to be targets of viral interference.[13] Viruses such as MCV, EBV and HPV can affect the initiation phase of translation.[13] Some tumor viruses encode proteins that inhibit the translation inhibitory kinase PKR signalling and promote autophagy such as EBV BILF1, KSHV viral interferon regulatory factors 2 and 3 (vIRF2/3) and HCV non-structural protein 5A.[13] PKR phosphorylates the subunit of the eukaryotic translation initiation factor 2 (eIF2), leading to inhibiting of translation and stimulation of autophagy.[13]These reports point towards a plethora of mechanisms employed by tumor viruses to hijack and overtake the host translational machinery for their own survival and spread.[13]

Nucleolar functions and ribosome biogenesis

Nucleolus is regarded as the primary site for rRNA synthesis, which plays a major role in ribosome biogenesis.[13] Of late, several new functions have been assigned to the nucleolus, including activities such as cell-cycle regulation, gene silencing, senescence, innate immune response and stress sensing.[13] Viral proteins change nucleolar dynamics in two ways: first, these get localized into nucleolus and regulate nucleolar export of viral mRNA required for efficient replication and infection. Secondly, they affect redistribution of nucleolar proteomics.[13] The nucleolar modification could also have a major impact on Pol I-mediated transcription.[13]

Exosome pathway

The discovery of exosomes which are tiny vesicles secreted out of most cells, containing bioactive information, has attracted attention of not just cell biologist, but also virologist alike as this secretory pathway seems quite susceptible to viral manipulation.[14] The secretion of viral oncoprotein was found to be mediated by exosomes.[14] Further, exosomes secreted by EBV-infected cells also carry viral miRNAs called 'binder of Arl two' (BART) miRNAs, whose functional transfer to dendritic cells down-regulates CXCL11/ITAC, an immune-regulatory gene involved in immune suppression in EBV-associated lymphomas.[14]

Ubiquitin proteasomal system

Ubiquitin proteasomal system (UPS) regulates the intra-cellular stability and activity of proteins in cells by post-translationally attaching ubiquitin moieties to proteins.[13] While ubiquitination of protein is mediated by E3 ubiquitin ligases, deubiquitination is catalysed by a set of proteases called 'deubiquitinases' (DUBs).[13] Viruses often modulate or adopt the activity of E3 ubiquitin ligases or deubiquitinases to meet their requirements.[13] Therefore, Ubiquitin

proteasomal system (UPS) is now considered as an important target for Therapeutic intervention for various diseases.[13]

FACTORS CONTRIBUTING TO VIRAL CARCINOGENESIS:

Factors that induce DNA damage in infected cells are as follows:

Inflammation and DNA Damage Induced by Virus Itself:

Inflammation is a primary immune response to infection by pathogens. This process involves activation and directed migration of leukocytes from the venous system to the sites of infection in which tissue mast cells play a significant role.[15] A family of chemokines attract leukocytes, whose persistence at an inflammatory site is important in the development of chronic disease. Inflammation is referred to as a cancer "promoter", because it induces cell proliferation, recruits inflammatory cells, increases cellular levels of ROS, thereby, leading to oxidative DNA damage, and reduced DNA repair.[15] The deregulation of cell death and/or repair programs result in DNA replication and proliferation in chronically inflamed tissue.[15] Inflammation also causes resistance to apoptosis, secretion of pro-angiogenic and immunosuppressive factors, invasion and metastasis.[15] All of these processes contribute to carcinogenesis.

Inflammation and DNA Damage induced by Co-Infections

The virus does not destroy the cells it infects, thereby avoiding triggering of inflammation. Co-infections triggering inflammation can be of either viral or bacterial origin.[15] For example Co-infections with certain sexually transmitted diseases (STD) cause cervical inflammation and increase the risk of cervical cancer in HPV-infected women.[15] Furthermore, high levels of inflammatory mediators, such as cyclooxygenase (COX)-2, an enzyme responsible for prostaglandin formation, is observed in cervical cancer.[15] The inflammation induced by these co-infections can induce the generation of reactive oxygen species (ROS), which can contribute to carcinogenesis by damaging DNA.[15]

Oxidative Stressors, Viruses and Cancer

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are charged free radicals that are primarily generated in mitochondria as by-products of aerobic respiration, cytochrome P450 activity and peroxisome function.[15] In normal conditions, the pro- and anti-oxidant systems maintain ROS homeostasis. A lack of proper balance between these two sets of systems result in changes to cellular levels of ROS and can lead to oxidative stress (OS).[15] In general, the sources of OS can be divided into two broad categories: exogenous and endogenous. Endogenous OS, as discussed above, is primarily derived from natural processes, such as cellular signaling, metabolic processes and inflammation.[15] Exogenous and environmental sources include ionizing radiation, such as X-, γ- and cosmic rays, α-particles from radon decay, oxidizing chemicals and UVA solar radiation.[15] For example, ionizing radiation generates radicals, including superoxide, hydrogen peroxide and hydroxyl radicals, most of which are generated during the radiolysis of water.[15] Of these, the hydroxyl radical is the most damaging species and produces mostly single-strand breaks. Overall, radiation induces genetic instability and chromosomal rearrangements, and many of these rearrangements are similar to those found in human cancers.[15] Chronic exposure to viral infections also induce the constant generation of free radicals, which can damage cellular biomolecules, including DNA.[15] DNA damage produced by OS results in apurinic/apyrimidinic (abasic) DNA sites, oxidized purines and pyrimidines, and single- and doublestranded DNA breaks.[15] Therefore, the ROS- and RNS-induced oxidative and nitrative DNA damage that frequently occurs during inflammation can contribute to carcinogenesis.[15]

DIAGNOSIS OF VIRAL INDUCED CARCINOGENESIS

Despite development of novel therapeutic methods in recent years, prognosis of advanced cancer remains very poor. Major risk factors for cancer are chronic infections as a result of viruses and exposure to various exogenous carcinogens.[16] Molecular approaches has recently revealed involvement of altered *genes in carcinogenesis*. During the past decade, advances in diagnostic technology and other DNA signal and target amplification techniques have resulted in molecular diagnostics becoming key procedures. Such techniques are conceptually simple, highly specific, sensitive, and amenable to full automation.[17] Diagnostic virology is rapidly moving into the mainstream of clinical medicine as a result of the convergence of several independent developments. [17] Also, viral diagnosis may be important for public health purposes and in analysis of virus associated cancers. Viral isolation and a number of methods for detection of viral antigens, nucleic acids, and antibodies (serology) are the core repertoire of techniques used for the laboratory diagnosis of viral infections.[18]

Methods Used in Diagnostic Virology[18]

1. Cell culture
2. Antigen detection
   - -Fluorescent antibody staining
   - -Immuno-peroxidase antibody staining
   - -Enzyme immunoassay
3. Nucleic acid detection
   - -Polymerase chain reaction
   - -Other nucleic acid amplification methods
4. Electron microscopy
5. Cytology
6. Histology
   - -Immunohistochemistry
   - -In situ hybridization
7. Serology

Cell culture:

The modern era of diagnostic virology dates to the first descriptions of viral isolation in cell culture by Weller and Enders in 1948 and Enders et al. in 1949.[18] Indeed, the need for cell culture techniques is the raison d'être for virology laboratories as entities separate from other general clinical microbiology laboratories.[18] While the relative importance of viral isolation as a diagnostic method is rapidly diminishing, it still remains necessary because it is the only technique capable of providing a viable isolate that can be used for further characterization, such as with phenotypic antiviral susceptibility testing.[18] An additional advantage is that in contrast to most antigen and nucleic acid detection methods, viral culture allows detection of multiple viruses, not all of which may have been suspected at the time the culture was ordered.[18] Because no specific cell culture type can support the growth of all medically relevant viruses, virology laboratories must maintain several different cell culture types.[18]

Antigen detection:

Methods of antigen detection include fluorescent antibody (FA) staining, immuno-peroxidase staining, and Enzyme immunoassay (EIA).[18] Of these, FA staining is the most widely used in diagnostic virology.[18] Rapid viral diagnosis by means of FA staining was first described by Liu in 1956 for detection of influenza and was pioneered for numerous viruses by Gardner and McQuillan.[18] The method was widely adopted by clinical laboratories during the 1980s, particularly for detection of respiratory viruses.[18] The commercial availability of specific monoclonal antibodies was crucial.[18] Antigen detection methods are particularly useful for viruses that grow slowly or are labile, making recovery in culture difficult.[18]

Nucleic acid detection:

The development of PCR analysis in 1985 made it possible to diagnose viral infection through sensitive detection of specific viral nucleic acids.[18] Any virus can potentially be detected in this way, and applications of PCR analysis and other nucleic acid amplification techniques continue to be developed.[18] There is little doubt that during the next decade, applications of nucleic acid detection techniques will drastically reshape the field of diagnostic virology.[18] By inclusion of a step employing the enzyme reverse transcriptase (RT), PCR analysis can be adapted to detect viral RNA.[18] Nucleic acid amplification assays can be classified as target amplification assays or signal amplification assays.[18] Examples of target amplification assays in addition to PCR include the ligase chain reaction, which has been used for detection of sexually transmitted disease agents, and the transcription-mediated amplification assay.[18] In signal amplification assays, the target itself is not amplified; rather, amplification is of a chemical signal used to detect hybridization of a probe with the target nucleic acid.[18]

Electron microscopy:

EM was first developed in the 1930s, by physicists in various countries, including Germany, in particular (reviewed in Haguenau et al., 2003).[19] The first microscope for TEM (transmission electron microscopy), which was also known as a 'supermicroscope', was initially described by Max Knoll and Ernst Ruska in 1932 (Knoll and Ruska 1932; Ruska 1987).[19] This microscope had a much higher resolution than the light microscopes of the time, and promised to revolutionize many aspects of cell biology and virology.[19] In research,

new imaging techniques for fluorescence light microscopy have supplanted TEM, making it possible to study live cells and dynamic interactions between viruses and the cellular machinery.[19] It is very useful for the initial identification of unknown viral agents in particular outbreaks, and is recommended by regulatory agencies for investigation of the viral safety of biological products and/or the cells used to produce them.[19]

Cytology:

In recent years, cytopathologists have observed that the alterations in cell morphology that accompany certain virus infections can be recognised in Papanicolaou smears submitted to routine examination by light microscopy.[20] The cytopathic changes due to herpes simplex virus, varicella-zoster virus, cytomegalovirus, and the human polyomaviruses have been described in epithelial cells in cytological specimens such as cervical smears, urine, sputum, and conjunctival scrapes, and the presence of virus infected cells in the smears has been shown to be a convenient and reliable guide to active infection of the patient with these viruses.[20] These observations have widened the scope of the cyto-diagnostic technique for they imply that the large numbers of cytological specimens that are submitted routinely for examination for malignant cells can at the same time be screened for evidence of viral activity.[20]

Histology:

Viruses induce characteristic morphologic changes during their replication in the cells they infect.[21] Morphologic features indicative of a viral infection include the formation of inclusion bodies in the host cell nucleus, cytoplasm or both.[21] For some viruses, formation of multinucleate giant cells, presence of a perinuclear halo around the infected cell, lymphocytic infiltration or even cellular necrosis can be useful.[21]

Immunohistochemistry:

Immunohistochemistry (IHC) continues to be one of the main adjunctive methods to conventionally stained sections in histopathology.[22] This is mainly related to the fact that it is a relatively simple, fast, and inexpensive method. Because of technical advances, there has been a significant increase in the number of diagnostic immunohistochemical stains available for pathologists and dermatopathologists in recent years.[22] Viruses are separated into families on the basis of the type and form of the nucleic acid genome, of the morphological features of the virus particle, and of the mode of replication.[22] There are 4 important families involved in cutaneous viral diseases: the DNA families of Herpesviridae, Papillomaviridae, and Poxviridae, and the RNA family Picornaviridae.[22] Two of the most specific histopathologic findings are the presence of koilocytosis, which is not invariable, especially in long-standing warts, and the so-called koilocytotic atypia, mostly seen at the granular layer, which consists of nuclear variation in size and staining pattern, with irregularity of nuclear membrane and binucleated or multinucleated cells.[22] Papillomavirus antigen can be detected by immunoperoxidase methods.[22] Immunohistochemical detection of the HPV L1 capsid antigen is indicative of replicative HPV infections, but this method does not facilitate identification of the virus type involved, either.[22]

Serology:

The diagnosis of viral induced cancer by detection of specific antiviral antibodies is a traditional method whose clinical utility is limited by the need for comparison of acute and convalescent antibody titers.[18] However, detection of virus-specific IgM antibodies allows a diagnosis to be made from a single specimen.[18] Viruses for which definition of immune status by serology is useful include VZV, CMV, EBV, HSV, measles and rubella viruses, parvovirus B19, hepatitis A (total antibodies), and hepatitis B (antibodies to the hepatitis B surface antigen).[18]

SUMMARY & CONCLUSION

Globally, almost 20% of cancers are related to infectious agents. Several viruses with oncogenic potential stimulate cell proliferation and cause tumors and cancer in animals and humans.[23] They act with different mechanisms depending on different host factors.[23] Despite their prevalence, public health importance, and suitability for immunoprophylaxis and targeted therapies, understanding and managing virus-induced cancers still faces formidable challenges.[24] This is due to limited animal models of disease, the disparate nature of viral-induced cancers, the very distinct types of viruses that cause them, and the complex nature of the virus-host cell interactions leading to cancer development.[24] Human viral oncogenesis has common traits: (1) oncoviruses are necessary but not sufficient for cancer development, so cancer incidence is much lower than virus prevalence in human populations; (2) viral induced cancers appear in the context of persistent infections and occur many years to decades after acute infection; (3) the immune system can play a deleterious or a protective role, with some human virus-associated cancers increasing with immunosuppression and others appearing in the context of chronic inflammation.[24]The tumor viruses with small genomes integrate into host cell chromosomal DNA and cause mutations and chromosomal rearrangements that predispose to cancer.[23] Many retroviruses do not have viral oncogenes.[23] They integrate near some of the proto-oncogenes, activate their expression by proviral insertional mutagenesis, and modulate growth and differentiation of the host cells.[23] Viral induction of tumors is the direct effect of a complex interaction of a single virus particle with the target cell.[25] However, even the effective entrance of the virus into the cell does not necessarily result in transformation.[25] Replication of the cell DNA may be required for integration of the viral DNA based on homologous areas, and the low efficiency of transformation suggests that this probably requires precise timing of multifactorial events.[25] Early stimulation of cell DNA synthesis by induction of increased DNA synthetic enzyme activity may increase the opportunity for integration.[25] Once integration has occurred, virus maturation is suppressed, but derepression of cell DNA synthesis continues.[25] Early viral coded proteins demonstrated as new antigens may play a major role in this derepression.[25]

The purpose in this communication is to utilize our current knowledge of viral carcinogenesis for the search for a viral etiology in human cancers, and to indicate what has been learned so far and what is currently being done to determine whether a virus may cause any human malignancy.

REFERENCES

1. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis. 2000; 21(3):405–26.
2. Bergonzini V, Salata C, Calistri A, Parolin C and Palù G. View and review on viral oncology research. Infectious Agents and Cancer. 2010; 5(11).
3. Zheng ZM. Viral Oncogenes, Noncoding RNAs, and RNA Splicing in Human Tumor Viruses. Int. J. Biol. Sci. 2010; 6(7):730-55.
4. Chen Y, Williams V, Filippova M, Filippov V and Hughes PD. Viral Carcinogenesis: Factors Inducing DNA Damage and Virus Integration. Cancers. 2014; 6:2155-186.
5. Mesri EA, Feitelson MA and Munger K. Human Viral Oncogenesis: A Cancer Hallmarks Analysis. Cell Host & Microbe. 2014; 15:266-82.
6. Pessach VM, Lotem M. Viral Carcinogenesis in Skin Cancer. Curr Probl Dermatol. Basel.2007; 35:39–51.
7. Devi PU. Basics of carcinogenesis. Health Administrator; 17(1): 16-24.
8. Sonnenschein C and Soto AM. Theories of carcinogenesis: An Emerging Perspective. Semin Cancer Biol. 2008; 18(5): 372–77.
9. Vineis P, Schatzkin A and Potter JD. Models of carcinogenesis: an overview. Carcinogenesis. 2010; 31(10): 1703–709.
10. Braoudaki M and Stathopoulou FT. Tumorigenesis related to retroviral infections. J Infect Dev Ctries 2011; 5(11):751-58
11. de Oliveira DE. DNA viruses in human cancer: An integrated overview on fundamental mechanisms of viral carcinogenesis. Cancer Letters. 2007; 247: 182–96.
12. de Oliveria AndradeLJ, D'Oliveira A, Melo RC, De Souza EC, Costa Silva CA and Paraná R. Association between Hepatitis C and Hepatocellular Carcinoma. J Glob Infect Dis. 2009; 1(1): 33–37.
13. Ahuja R, Jamal A, Nosrati N, Pandey V, Rajput P, Saxena N, et al. Human oncogenic viruses and cancer. Current science. 2014; 107(5): 768-85.
14. Meckes DG. Exosomal Communication Goes Viral. Journal of Virology. 2015; 89(10): 5200-203.
15. Nikitin PA and Luftig MA. The DNA damage response in viral-induced cellular transformation. British Journal of Cancer. 2012; 106: 429 – 35.
16. Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, et al. Genome-wide analysis of gene expression in Human Hepatocellular carcinomas using cDNA microarray: Identification of genes involved in viral carcinogenesis and tumor progression. Cancer Research. 2001; 61: 2129–137.
17. Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE. Multiplex PCR: Optimization and Application in Diagnostic Virology. CMR. 2000; 13(4): 559–70.
18. Gregory A. Storch. Diagnostic Virology. CID. 2000; 31: 739-51. 28.
19. Roingeard P. Viral detection by electron microscopy: past, present and future. Biol. Cell. 2008; 100: 491–501.
20. Coleman DV. Cytological diagnosis of virus-infected cells in Papanicolaou smears and its application in clinical practice. J Clin Patho. 1979; 32: 1075-89.
21. Gupta E, Bhalla P, Khurana N, Singh T. Histopathology for the diagnosis of infectious diseases. Indian J Med Microbiol. 2009; 27(2): 100-06.
22. Molina-Ruiz AM, Santonja C, Rütten A, Cerroni L, Kutzner H, Requena L. Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections—Part I. Cutaneous Viral Infections by Herpes viruses and Papilloma viruses. Am J Dermatopathol. 2015; 37:1–14.
23. ŞEVİK M. Oncogenic viruses and mechanisms of oncogenesis. Turk. J. Vet. Anim. Sci. 2012; 36(4): 323-29.
24. Mesri EA, Feitelson MA, Munger K. Human Viral Oncogenesis: A Cancer Hallmarks Analysis. Cell Host & Microbe 15. 2014; 266-82.
25. Habel K. The Biology of Viral Carcinogenesis. CANCER RESEARCH 28. 1968; 1825-31