Meeting highlights: National Cancer Institute workshop on Molecular Signatures of Infectious Agents

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1. Introduction

Viruses are associated with at least 20% of human cancer which is likely to be an underestimation of the actual contribution of viruses to human cancers [1–8]. These estimates are rather conservative because techniques employed to determine the prevalence were not as accurate as provided by more sensitive technologies [3,9–16]. The proportion of cancers attributed to infectious agents is higher in developing countries (22–23%) than in the developed countries [9,10]. The most common infectious agents associated with human cancer and their prevalence are shown in Fig. 1. Since in many cases the infectious agents are ubiquitous, the key is in identifying the subpopulation of exposed individuals who are likely to develop cancer as a consequence of the initial exposure to the infectious agent [9,17,18]. Identifying this subpopulation provides a potential solution to reduce the disease. Molecular markers should be developed that may distinguish between infections per se and those infections that are contributing to development of cancer. As such, it is not unreasonable to predict that to make such distinctions will require identifying differing patterns of expression of multiple markers, i.e., generating a molecular signature for specific cancer. These molecular signatures must permit the reliable and accurate identification of individuals-at-risk at an early enough stage in cancer development such that an intervention can be effective. The information gained from defining molecular signatures for cancers should provide potential diagnostic tools in addition to potential insight into the mechanisms of action of viruses in cancer. These studies may also define potential targets for new modalities of cancer therapy.

Although the biology of viruses has advanced significantly in the past three decades, our ability to detect them at earlier stages of tumor progression has been limited. In light of high throughput technologies, such as microarray and proteomics, it is likely that these technologies will also enable us to map out the molecular imprints on host tissues left by the invading viruses. The Cancer Biomarkers Research Group of the Division of Cancer Prevention, National Cancer Institute sponsored a workshop entitled "Molecular Signatures of Infectious Agents" in Bethesda, Maryland, September 7–8, 2000 to identify molecular signatures of infectious agents and utilize this information for risk assessment and development of prevention strategies against these infectious agents.

2. Objectives of the workshop

The specific objectives of the workshop were to review state-of-the-science in detection technology that can identify extraneous genomic insertion in human cancers and to establish future research directions for using the molecular signatures of infectious agents

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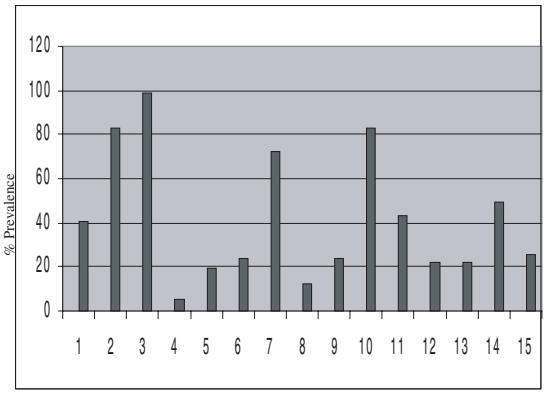
for early detection, risk assessment, and prevention of cancer. The workshop included presentations by invited scientists actively engaged in research on infectious agents and human cancer. Sessions included an overview of the infectious agents and their interaction with host, contribution of infectious agents in the development of disease, technology advancement in detecting multiple infections, role of growth factors and cytokines in cancer progression, animal models in understanding host-virus interaction, and development of prevention strategies against infectious agents. This commentary summarizes the highlights of the meeting and the recommendations made by the panel of experts in the areas of infectious agents, molecular detection technologies, and laboratory-to-field applications.

3. Contribution of infectious agents in the development of cancer

Dr. Paul Lambert, Professor of Oncology at the University of Wisconsin Medical School, presented the keynote address for the workshop. He presented information on viruses that appear to be associated with cancer, such as human papilloma virus (HPV) in cervical cancer. Cervical cancer is the second most prevalent cancer in females in the world. Involvement of different strains of papilloma virus has been demonstrated in cervical cancer. HPVs are small DNA tumor viruses that have a stringent tropism for epithelial cells. Specific genotypes of HPVs (e.g., HPV-16, HPV-18, HPV-31, HPV-33) that infect the anogenital tract are also seemingly involved in the etiology of malignant lesions, most notably cervical carcinoma. Cervical intraepithelial neoplasia (CIN), benign cervical lesions that are the progenitors of cervical carcinomas, are frequently detected in women infected with high-risk HPVs. CIN is usually graded I through III, depending on increasing degree of atypicality and resemblance to cancer. The integration of viral DNA into the host genome causes increased expression of two viral transformation genes, E6 and E7. These genes assist in oncogenic process by inactivating p53 and retinoblastoma tumor suppressor genes [19]. The tumor suppressor gene pathway is a common mechanism involved in host-virus interaction (Fig. 2). The contribution to carcinogenesis progression was described for an animal model using transgenic mice. He described the role of genetics in determinating progression to cancer and the interactions between various molecular targets of the HPV virus. Results from ongoing research indicate that there are multiple mechanisms for tumor initiation and promotion. This is the case in HPV-16, in which two viral genes, E6 and E7, are commonly expressed in human cervical cancer cells. In a study in transgenic mice, E6 acts sparingly at the promotion stage but strongly at the progression stage of tumor growth. The E7 gene acts in the opposite manner [20]. These findings highlight the importance of identifying genes of infectious agents in development of cancer to determine their specific role at each stage of cancer development [20–22].

Dr. Laimonis A. Laimins, Professor in the Department of Microbiology-Immunology, Northwestern University Medical School, discussed changes in cellular expression induced by HPV and their association with high risk genotypes (i.e., HPV-31) that include tumors and low risk genotypes (HPV-11) that induce benign lesions [23]. Microarray analysis allowed investigation of transfected keratinocytes and identification of genes activated by HPV-31 gene products [11,24]. Results from his laboratory indicated that of approximately 7,200 genes investigated, about 170 genes were activated, although no particular pattern emerged to account for the effect of the virus. However, genes repressed by HPV-31 included distinct families of genes, such as the interferon inducible genes, which may play a role in immune susceptibility by reducing the protein Signal Transducer and Activator of Transcription (STAT-1). Dr. Laimins compared the expression of genes by HPV-31 and those expressed by HPV-11. A number of viral genes interact with the host and involve in the development of the disease (Table 1).

The current methods used for the detection of HPV are either antibody (directed against viral oncoprotein E6 or E7) based or PCR based. HPV can exist as an episome (circular viral genome present in the cytoplasm) or integrate in the host genome [18,19, 25]. Dr. Magnus von Knebel Doeberitz, Professor of Molecular Oncology at the University of Heidelberg, Germany discussed a PCR assay for the amplification of papillomavirus oncogene transcripts (APOT) [26]. APOT may have an application in identifying a specific progression marker in future cervical cancer screening protocols. Primers for detecting episomal or integrated HPV genome are well-characterized and used on clinical samples. Dr. Elizabeth R. Unger, Acting Chief of the Human Papillomavirus Section, Centers for Disease Control and Prevention (CDC), provided background data on the role of HPV in cervical cancer.



	Cancer	Infectious Agent
1	African Burkitt Lymphoma	Epstein Barr Virus
2	Nasopharyngeal Carcinoma	Epstein Barr Virus
3	Cervical Cancer	Human Papilloma Virus
4	Schneiderian Papilloma	Human Papilloma Virus
5	Head and Neck Papilloma	Human Papilloma Virus
6	Liver Cancer	Hepatitis B Virus
7	Lymphoma	Hepatitis Virus G
8	Breast Cancer	Polyoma Virus
9	Breast Cancer	Epstein Barr Virus
10	Kaposi Sarcoma	Human Herpes Virus-8
11	Hematologic Neoplasia	HHV-6
12	T cell Leukemia	HTLV
13	Myeloid Leukemia	Fungus
14	Oral carcinoma	Human Papilloma virus
15	Colorectal carcinoma	JC Virus

Fig. 1. Prevalence of infectious agents in cancer.

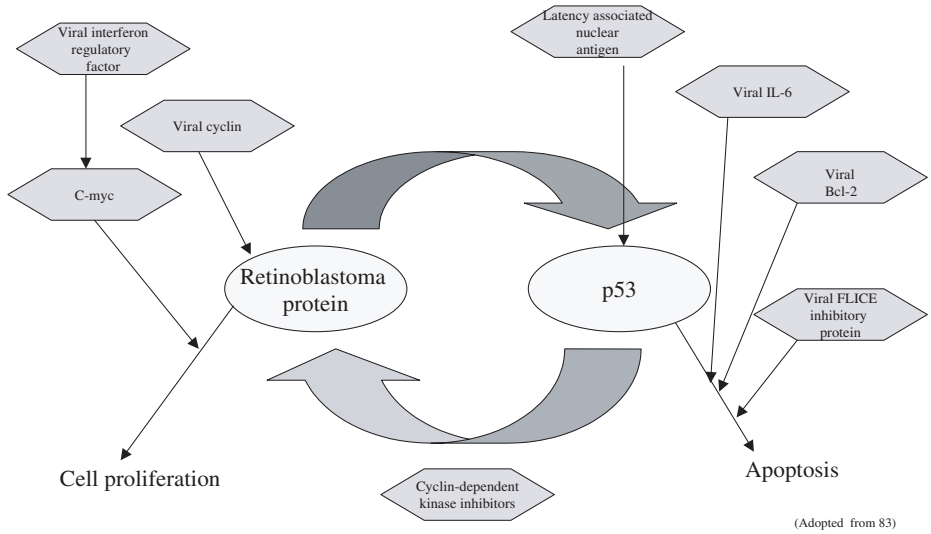


Fig. 2. Viral proteins that interact with the tumor-suppressor pathway governed by retinoblatoma protein and p53.

4. Known versus novel agents

Dr. Lambert emphasized that a likely deficit in our knowledge of cancer etiology is in identifying the role of existing and novel viruses in cancers which today are not recognized to be virally caused. There are numerous examples today of human cancers for which a viral etiology has been proposed but not established [12]. For instance, the controversial role of SV40 and human polyomaviruses in CNS (central nervous system) tumors, the recently raised potential role of EBV (Epstein-Barr virus) in breast cancer, and the argued role of TTV in liver cancer need further investigation [27–30]. It is reasonable to speculate that other human cancers are caused by viruses, and that some of these viruses may not yet be identified. Molecular signatures for virally-associated cancers, as part of a comprehensive program encompassing epidemiological research, basic virology and cancer biology, may help us identify existing and/or novel viruses that contribute to additional human cancers. For example, if it were to be discovered that human papillomaviruses (HPV) cause specific changes in cervical cancer cells as they progress towards cancer, and were these changes found to reflect the function of virally encoded gene products, then it would be reasonable to look for similar changes in cancers at other anatomical sites in which HPV is suspected but has not been proven to be a

Table 1
Viral genes involved in transformation

Viral gene	Referemce
E1	24
E6	18
E7	18
Cyclin	83
Bcl2	5, 6
IL-6	83
FLICE Inhibitory Protein	83
LANA	83
Rb	83
Interferon	11
p53	83
Tax	40–44
Tat	2
HBx	47

causal agent (e.g. certain skin cancers, oral cancers). Many oncogenic viruses appear to target a similar set of cellular targets, leading to their inactivation and/or deregulation. For instance, HPVs, SV40, Py, and adenoviruses all target the Rb and p53 tumor suppressor genes, as well as p300/CBP (Fig. 2). The combined effect of these disruptions is likely to result in certain alterations in the pattern of gene expression and function. Furthermore, these alterations arise in the absence of mutational events that otherwise occur in cancers where viruses are suspected to contribute. These differences potentially could be exploited to identify cancers caused by unknown or unappreciated oncogenic viruses which act through a similar mechanism.

5. Proteomics approaches to identify molecular signatures of infectious agents

Dr. Timothy M. Block, Director and Professor at The Jefferson Center, Thomas Jefferson University, discussed the use of two-dimensional gel electrophoresis (2-DE) as a tool for the systematic analysis of polypeptides of hepatitis B (HBV) and hepatitis C (HCV) viruses. Hepatitis virus B (HBV) infection is a major health problem because there are 300 million carriers worldwide. Nearly 50% of the population in parts of Africa and southeast Asia have been infected with HBV [5,6]. There are about 300,000 new cases per year in the USA and 0.5% of the US population are carriers of HBV [31]. Chronic active hepatitis is found in 25% of the carriers. HBV is a precursor to cirrhosis of the liver and is associated with an increased lifetime risk of developing primary hepatocellular carcinoma (HCC), usually 20–30 years after infection with the virus. Many HCC cells contain portions of the HBV genome integrated into their chromosomes, but there is no common viral gene or product that is persistently expressed. HBV may also synergize with environmental factors such as alcohol-associated cirrhosis in causing HCC. HBV vaccination initiatives designed to reduce perinatal morbidity and mortality are currently underway [9]. 2-DE is being used to identify serum polypeptides associated with the onset and progression of HBV and HCV. This proteomic approach could potentially be applied to other diseases.

Dr. Block discussed the incidence, mortality, and pathology of HBV and HCV and their role in liver cancer. Because HCC has a long latency before disease is apparent, there is an unusually long period when prevention of HCC is possible. He explained state-of-the-science in the field of therapeutics for the hepatitis viruses, such as glucosidase inhibitors, which seem to be virus-specific and have a minimal cytotoxic effect on the host cell. Proteomics research in his laboratory focuses on the identification of individuals infected with HBV and HCV who are likely to develop HCC. Dr. Block presented progress made in developing techniques and software that can identify biomarkers and stressed that these investigations must continue in order to refine the technique and confirm their importance in molecular signatures of infectious disease. The research on alterations in oligosaccharides in hepatitis infection has led to the development of "glycomics," which has promise as a new class of diagnostic markers. Dr. Block used serum from patients for protein analysis and purified hepatitis B-subviral particles for glycomics.

6. Molecular approaches to detect virus in brain and colon cancers

Dr. Kamal Khalili, Director of the Center for Neurovirology and Cancer Biology, Temple University, presented background material on JC virus (JCV). JCV has been associated with oncogenic properties through its T-antigen, which suppresses the genes p53 and pRb [32–35]. JCV is responsible for dysmyelination diseases of the central nervous system in immunosuppressed individuals [32–35]. Dr. Khalili explained the possible role of JCV in human tumors, including medulloblastomas, pilocytic astrocytomas, and oligoastrocytomas. Evidence of JCV T-antigen was evident in each tumor type, making JCV or its proteins potential molecular markers for human cancer. He presented results demonstrating β-catenin expression in

mouse and human medulloblastoma and suggested that β-catenin also might be a possible molecular marker for the development of tumors associated with JCV.

JCV has been reported in colon cancer also [13,36, 37]. Laghi et al. [13] have found T antigen DNA sequences of JCV in the mucosa of normal human colons, colorectal cancers, colorectal cancer xenografts raised in nude mice, and in the human colon cancer cell line SW480. A larger number of viral copies was present in cancer cells than in non-neoplastic colon cells, and sequence microheterogeneity occurred within individual colonic mucosal specimens. The improved yield of detection after treatment with topoisomerase I suggested that the viral DNA was negatively supercoiled in the human tissues. Taken in the context of the known biological properties of T antigen and recent epidemiological findings that link JCV to aneuploid "rogue" cells, it was inferred that infection with JCV is a prime candidate for a role in chromosomal instability, a phenomenon commonly found in colorectal cancers. Topoisomerase I treatment of viral samples (before PCR) is indispensable to release the negatively supercoiled JCV DNA in colon cancer patients.

7. Viral gene product as a transactivator and its role in DNA replication and repair

Dr. Susan Marriott, Associate Professor in the Division of Molecular Virology and Microbiology, Baylor School of Medicine, presented information on the human T-cell leukemia virus type 1 (HTLV-1) Tax protein, a transcriptional transactivator and viral oncogene [38]. HTLV is the first human retrovirus identified [39]. Adult T cell leukemia (ATL) predominates in the islands of Southern Japan, and retroviral particles were isolated from a cell line derived from an ATL patient. HTLV-1 is associated with adult T-cell leukemia and tropical spastic paraparesis, each characterized by infection of CD4+ T-cells. Each of these diseases occurs in only approximately five percent of infected individuals and can have a latency of 20–40 years. A Tax-reactivated gene, proliferating cell nuclear antigen (PCNA), is important in DNA replication and repair and is overly expressed in almost all tumors and transformed cell lines [40]. Tax reacts with cellular transcription factors, such as CREB (cyclic AMP response element binding proteins), NF-κb, SRF (serum response element binding factor). To date, 20 genes (involved in cell division and geneome instability) have been identified where Tax is involved [41–44]. As with EBV and lymphomas, it is likely that additional genetic and/or environmental factors participate with HTLV-1 in the pathogenesis of the ATL. Highly sensitive and specific PCR based methods are used for the detection of HTLV. Dr. Marriott described research showing that Tax suppresses DNA repair while stimulating DNA replication, thus contributing to the monoclonal nature of HTLV-1 transformed cells.

Dr. Betty L. Slagle, Assistant Professor of the Department of Molecular Virology and Microbiology, Baylor College of Medicine, presented information on the association of HBV and environmental carcinogens as primary risk factors for HCC [45]. In transgenic mice, it has been shown that a cofactor, HBx transactivating protein, is associated with the inhibition of DNA repair functions of human and murine cells damaged by either ultraviolet light or aflatoxin B1 exposure [46]. One hypothesis is that HBx inhibits DNA repair in chronic HBV infection. The ATX mouse model has been used to show that HBx does inhibit DNA repair [47]. Dr. Slagle described experiments in transgenic mice that show the potential of this model in liver cancer and the possibility of better defining the role of HBx as a cofactor that is a tumor promoter.

8. Host immune response to infection

Dr. Jae Jung, Chairman of the Tumor Virology Division, New England Regional Primate Center, Harvard Medical School, described the mechanisms used by viruses to target and modulate various aspects of the host's immune system. Dr. Jung's research focused on human herpesvirus-8 (HHV-8) and Kaposi's sarcoma-associated herpesvirus (KSHV) [48–50]. Compelling epidemiologic evidence, including the peculiar geographical distribution of Kaposi's sarcoma, prompted speculation about an infectious cause as well as possibility of sexual transmission [51]. In 1994, Chang's group identified DNA fragments of a previously unrecognized herpesvirus, which has been called Kaposi's sarcoma-associated herpesvirus in a Kaposi's sarcoma skin lesion from a patient with AIDS [52]. Over 95% of Kaposi's sarcoma lesions, regardless of their source or clinical subtype, have been found to be infected with KSHV [53]. Although studies have been published on the contribution of cytokines as well as HIV tat protein to the pathogenesis of Kaposi's sarcoma lesions, it has been demonstrated that the presence of KSHV is the primary and necessary factor in the development of this tumor. In addition, immunosuppression of the

host appears to be an important cofactor in the clinical expression of Kaposi's sarcoma in some KSHV-infected patients. Dr. Jung described the synergistic effects of B7-2 and ICAM-1, ligands of the natural killer (NK) cell-mediated cytotoxicity receptors, and the manner in which they are downregulated by KSHV. This may be the primary mechanism for infection. K3 and K5 proteins are zinc-finger membrane proteins encoded by KSHV and are significant inhibitors of immune response; for example, K5 suppresses the B7-2 and ICAM-1 surface expressions and allows the KSHV to avoid NK immunity [53]. HHV-8 DNA has been detected in essentially all Kaposi's sarcoma (KS) lesions investigated, including those associated with transplantation. However, the possibility of HHV-8 detection in serum before appearing in the tumor is unknown. Like other viruses, KSHV is also detected by PCR based methods [54].

9. Are animal viruses human pathogens and do they cause transformation?

Dr. Robert L. Garcea, Professor of Pediatrics at the University of Colorado School of Medicine, presented an overview of simian virus 40 (SV40) and discussed the possibility that it may be a human pathogen [55–57]. SV40 genomic sequences have been found in various human tumor samples, including choroid plexus neoplasms, ependymomas, osteosarcomas, and mesotheliomas, and is virtually identical to that of the human polyomaviruses BK and JC [55,58]. Dr. Garcea described regions of the SV40 genome that are similar to other human viruses and reviewed the theory that the virus may be associated with infection through human polio vaccines in the 1950s and 1960s. He described the findings from PCR investigations as a base for the evolving theory that SV40 may be a human pathogen. Dr. Garcea recognized that this area is controversial but may be valuable to investigate because recent research results and advanced laboratory techniques make it possible to answer this question. He listed research results in support of SV40 as a human pathogen and results that dispute the premise.

10. Multiple infections

Epstein Barr Virus (EBV) has been associated with lymphoproliferative disorders in individuals with immune dysfunction [59]. Most of these disorders are polyclonal B cell lymphoproliferations. Genes responsible for the lytic cycle are generally silenced or expressed at very low level in lymphoblastoid cells. Different ethnic groups with a high human leukocyte antigen (HLA)-A11 prevalence have been shown to experience a high rate of EBV infection, EBV-associated malignancies, and Epstein-Barr nuclear antigen (EBNA)-4 mutations. Recently, EBV infection has been reported in breast and cervical cancer; and HPV16 and EBV infection in Kaposi's Sarcoma [3,27,54,60,61]. EBV type A is more prevalent in western countries whereas type B in African countries. The epitopes 399–408 and 416–424 of EBNA-4 are major antigenic epitopes that elicit HLA-A11 cytotoxic T lymphocyte (CTL) response to EBV infection. Mutations selectively involving one or more nucleotide residues in these epitopes affect the antigenicity of EBNA-4, because the mutant EBV strains are not recognized by the HLA-A11-restricted CTLs. In EBV infected cells, p53 expression is low and Bcl_2 expression (apoptosis inhibitor) is high. PCR based techniques are currently used for detecting multiple infections.

11. Nonpathogens and human cancer

Some nonpathogens have also been associated with human cancer [6,62]. Helicobacter pylori prevalence is as high as 80% in cases of gastric cancer. The general consensus is that H. pylori acts as a cofactor in the development of stomach cancer [63,64]. There is some evidence that H. pylori infection increases with age. Schistosomes in bladder cancer and gram negative anaerobes have been found in colon cancer [65]. How much they contribute in the development of the disease is not well understood. Microscopic examination and PCR techniques are the methods of choice to detect these organisms. The successful demonstration in several mouse models that various H. pylori vaccines can induce not only protection against infection, but also regression of infection and associated lesions, raises the hope of the development of similar vaccines in humans. However, the relatively poor results so far obtained in other animal models more relevant to humans, such as cats and monkeys and in phase I–II human trials, suggest that much research is still needed. This involves the identification of suitable antigens, adjuvants and/or delivery system and routes of administration. The sequencing of the whole genome of H. pylori could help in the identification of appropriate antigens, but much still has to learned about the mechanisms

of protective immunity and therapeutic immunization. Although viruses are the major infectious agents, some fungi and bacteria are also associated with cancer [4, 63–65].

12. Current status of detection technologies

There has been a significant advancement in the development of detection technology during the last decade [3,4,11–16]. It was dueto nucleic acid based technology that TTV (TT virus) was isolated without prior knowledge of its sequence. Now this virus has been reported to be associated with hepatitis and HCC [67,68] though, as of yet, a causal relationship of TTV with these diseases has not been established. Similarly, protein-based techniques, generally called proteomics, have been developed to identify infectious agents [69,70]. Proteomics refers to the systematic analysis of polypeptides expressed by a given tissue, cell or species [69,71,72]. Protein are either isolated from serum or tissues for further analysis and detection of viral specific proteins. Advances in the standardization of 2-DE coupled with image analysis now permit the reproducible resolution of more than 1,000 polypeptides per run [73]. Further analysis of the resolved polypeptides, including identification and characterization of post translation modification identification is also possible [74]. Research is underway to test the hypothesis that changes in the amount or modification of serum polypeptides correlate with the onset of HCC or hepatitis [74–76]. Individuals chronically infected with hepatitis B or C virus (HBV, HCV) are at high risk for the development of HCC and hepatitis, with disease progression occurring relentlessly after many years [77]. Serum polypeptides from individuals at different stages in the disease continuum can be resolved by "Proteomic" 2-DE [78]. The proteomic approach is expected to liberate us from the need to "cherry pick" or "guess" the best biomarkers and let the data tell us which are the best markers of disease and virus infection.

13. Prevention strategies

The realization that approximately one-fifth of all cancers can be attributed to infectious agents opens a great prospective for the prevention and treatment of cancer [7,79]. This is particularly true for cancers of the cervix, stomach, and liver which are very common in developing countries, where they represent 91% of the cancers associated with infectious agents [6]. In terms of prevention, prophylactic vaccines (which generate neutralizing antibodies) and therapeutic vaccines (which induce cellular immune response) are being developed for a number of infectious agents discussed during the workshop. DNA vaccines generally induce strong cellular immune response. HBV prophylactic vaccine is already showing promising results as HBV vaccination prevents against HBV-related liver cancer many years later [6]. Developmental research on HPV vaccines to protect against cervical cancer is also progressing well. Vaccines against HCV, HTLV-1 and EBV are also in various stages of development, as are vaccines against HHV-8 [5]. Oncogenic viral infections are not the only targets of prophylactic vaccines. Intensive efforts are being made to develop preventive vaccines against H. pylori and Schistosomes, which are responsible for an enormous burden of disease, potentially including cancers of the bladder, colon, and liver [5]. Some other cancer prevention approaches against infection include antisense ribonucleotides and antisense ribozymes (with or without inducible promoters) which are very specific for their targets [80]. Transgenic animal model systems have been very instrumental in these kinds of approaches [17]. In addition, a pDNA (plasmid DNA) based vaccination strategy was found to be useful to fully protect from the outgrowth of tumors expressing EBV gene EBNA-4 [81]. Antiretroviral therapy has been tried for prevention of Kaposi's sarcoma [82]. Whether infectious agents cause the disease or not but developing vaccines against these agents will be beneficial to stop further infection.

Biological agents such as interferon alpha are now considered first-line therapy for some patients with epidemic cutaneous Kaposi's sarcoma. Subcutaneous, intravenous, or intralesional interferon alpha, all resulted in remissions in 20–60% of patients studied – results that were similar to those for single agent chemotherapy. Response rates correlated with base-line CD4 counts and the use of antiviral therapy [83].

The accumulating evidence indicate that viral and other infectious agents contribute directly or indirectly to a significant fraction of human malignancies worldwide. With the new tools of molecular biology and genetics, several new oncogenic viruses may be identified and the interaction of known and yet to be discovered agents will be defined. The ultimate legacy of the field will be the insights gathered into a fundamental understanding of the origins of cancer and in better treatment and even prevention of some cancers. There is no one

" human tumor virus", but there are many viruses capable of playing either some type of co-factor role or predominant causative role in the origin of many human cancers.

14. Recommendations

After two days of deliberations, the workshop participants recommended the following interrelated areas in need of support in developing molecular signatures for virally-induced cancers.

14.1. Define molecular signatures through gene expression profiling of normal, precancerous and cancerous lesions (*brute force screening*)

Gene expression profiling provides a powerful means for defining molecular signatures for diseases. Molecular signatures permit one to identify patterns of changes both at the RNA (gene chips/arrays) and protein (proteomics) levels between any given stage of cancer. They allow one to screen effectively for changes regardless of the level of knowledge that exists regarding the viral mechanisms of action in cancer. The success of this approach is based upon the availability and quality of the samples being compared. Human samples are optimal, where available; however, for many human cancers laboratory animal models or tissue culture models may be more practical sources of reproducible materials for analysis.

Cancer-associated viruses likely act, at least in part, in predisposing the cell to genetic changes, resulting in the progressive steps that lead to cancer growth. It may be beneficial, therefore, to identify early mutational changes in the progression to cancer. While involving primarily a distinct set of technologies from that used in developing molecular signatures, the knowledge gained from generating a genetic profile of a cancer and its precancerous lesions may help predict the molecular signature of these lesions.

14.2. Define molecular signatures of virus induced cancers through an understanding of the mechanism of action of viruses

While gene expression profiling approaches may be beneficial, the availability of matched tissue samples or validated animal models may limit their application. For many viruses, there is arising, through traditional reductionist scientific investigative efforts, fundamental insights into the mechanism by which they contribute to cancers. From these insights arise predictions for molecular signatures for virally-induced cancers. Thus, programs that test the validity of these predicted molecular signatures may provide great potential, especially where gene expression profiling studies are not feasible or as an augmentative approach to the latter.

14.3. Define host-immune responses that provide markers for progression of disease and susceptibility for disease

In all cases known, viruses that cause cancers are ones that persist for long periods in the host. How the host responds to the virus and how the virus modulates this response must be critical in allowing for viral persistence and indeed may be determinative of the risk of cancer. Both the general immune competence and the type of immune response (e.g. type I versus type II) may be determinative of the outcome of the viral infection and subsequent risk of cancer development. In some cases, as with HBV and perhaps also HCV, cancer may result from an underlying immunopathological disease. Specific haplotypes may confer risk for and protection from cancer; for instance, controversy surrounds assertions that haplotypes in class-II genes and extended haplotypes that include class-I-linked genes help determine risk of cervical cancer. In addition, oncogenic viruses, in many cases, have learned to evade the immune system; such evasion strategies likely contribute to their oncogenic potential. Therefore, the immunobiology of persistent viral infections, and in particular those that lead to cancer, must be better understood. This knowledge could contribute not only in helping identify patients at risk of developing cancer, but also in developing immunological intervention strategies.

14.4. Develop new/characterize existing animal models for virally-induced cancers

Validated laboratory animal models for virally-induced human cancers will be pivotal to the success of a molecular signatures program. For some cancers, lab oratory animal models may be essential in providing the critical tissue samples with which to identify molecular signatures, not only for the frank cancer but more importantly, the precancerous lesions that for some human cancers are difficult to identify/obtain. Animal models will also provide the means for testing the validity of a molecular signature, in understanding the un-

derlying mechanism of action by which the viral agent contributes to the molecular signature, and in providing a means of testing new therapeutic modalities for intervening in virally induced cancers that might arise as a consequence of defining molecular signatures. Thus, a molecular signatures program may need to encompass the development and use of validated animal models for virally-induced human cancers.

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