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Review Article

Viral oncology and development of preventive vaccines

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CONTENTS

Abstract

Viruses can be considered as parasitic genetic elements that need to infect cells to replicate. Cell proliferation is a major requirement to support viral replication. To this end, viruses have acquired the ability to induce the proliferation of infected cells via various mechanisms. In addition, many viruses can establish a transformed cell phenotype in *in vitro* systems and form tumors in animal models. More importantly, certain viruses are associated with some human malignancies. These viruses comprise human papillomavirus (HPV) and human hepatitis B and C viruses (HBV and HCV, respectively). According to a conservative estimate, 15% of cancers that are newly diagnosed worldwide have an infectious etiology. On these grounds, immunization programs against tumor-causing infectious agents might considerably reduce the incidence of cancer. To date, preventive vaccines have been developed against HBV and HPV.

Introduction

Viruses must infect cells, as their replication requires specific cellular genes that act in concert with viral factors. Viral-encoded genomes do not allow for the replication of the organism *per se*. On these grounds, viruses can be considered a sort of parasitic genetic element that must be transmitted to certain target cells, which then provide the environment to harbor the virus and support its replication program.

In many cases, mammalian viruses need to stimulate the proliferation of infected cells, which in turn generate those cellular factors that are required to sustain viral replication. To this end, viruses have devised various means to induce host cell proliferation (1). For instance, retroviruses may accidentally either activate, or at least overexpress, cellular genes involved in the induction of the proliferation program. This phenomenon can be caused by a random insertion of the retroviral genome into the host chromosomal DNA (2-4). Each retroviral genome carries two long-terminal repeats (LTRs) that encode for a viral enhancer and a viral promoter sequence. One LTR is located at the 5'-end of the retroviral genome and has the function of transcribing the retroviral messenger (m)RNA, whereas the second LTR is situated at the 3'-end of the retroviral genome. The presence of the 3'-LTR is a major requirement for retroviral biology and comes into play in the reverse transcription mechanism (4, 5). In addition, both LTRs are required for retroviral integration into the host cellular DNA (4, 5). However, the 3'-LTR could also activate the expression of a cellular oncogene, if this is proximal to the retroviral integration site (4). Such an event is termed insertional mutagenesis (2-4) (Fig. 1).

Another mechanism for activation, or overexpression, of cellular oncogenes is via an interaction between the retroviral enhancer and a certain endogenous oncogene promoter (4-6) (Fig. 2). This second mechanism was revealed by follow-up studies on the development of a hematological malignancy observed in 2 children who participated in a gene therapy clinical trial conducted in France in 2000 (6, 7). This clinical trial dealt with the treatment of severe combined immunodeficiency (SCID)-X1 and relied on retroviral-mediated gene transfer to express the correct form of the defective gene (7). In these cases, the retroviral enhancer stimulated the transcriptional activity of the endogenous promoter of the *LMO2* oncogene (6), the overexpression of which is associated with pediatric leukemias (8).

Other RNA or DNA viruses have the ability to directly stimulate cell proliferation in order to favor DNA synthesis. Such a direct stimulation of cell proliferation can be achieved via viral components that are either homologous to cellular growth factors or receptors for growth factors, or downstream agents of cellular growth factor signaling systems (1). For instance, Epstein-Barr virus (EBV) encodes for latent membrane protein (LMP)-1, which is homologous to tumor necrosis factor receptor (TNFR) (9-12). Polyomavirus middle T antigen is homologous to the receptor tyrosine kinase (1, 13). Another example is pro-

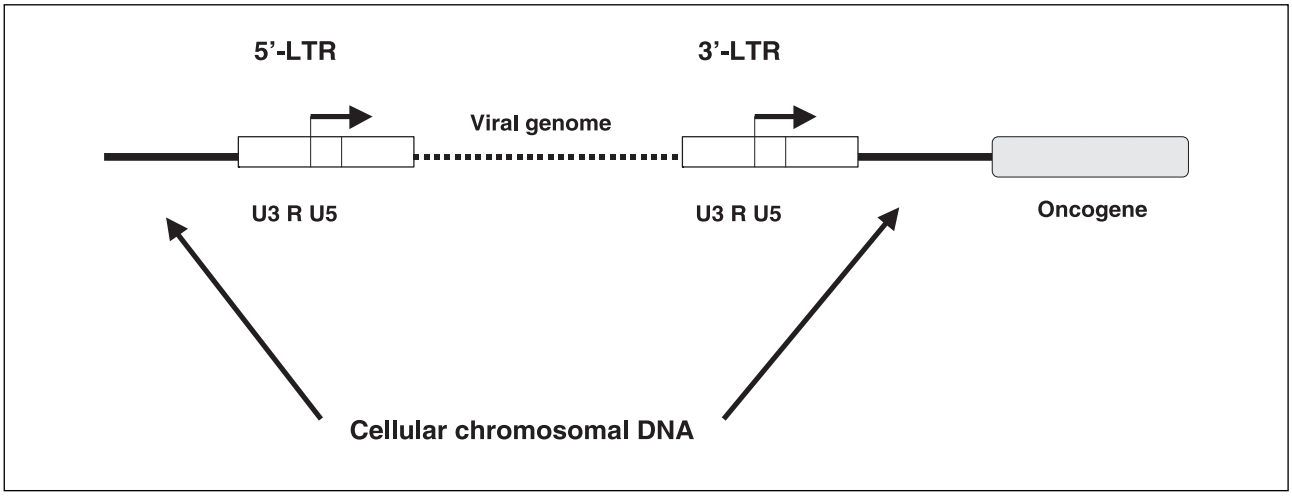

Fig. 1. Scheme of insertional mutagenesis. A retroviral genome is integrated into cellular chromosomal DNA. The 5'-LTR drives the expression of retroviral mRNA, whereas the 3'-LTR may activate the expression of a proximal cellular oncogene. This interaction usually has a short range of action. LTR, long terminal repeat; mRNA, messenger RNA.

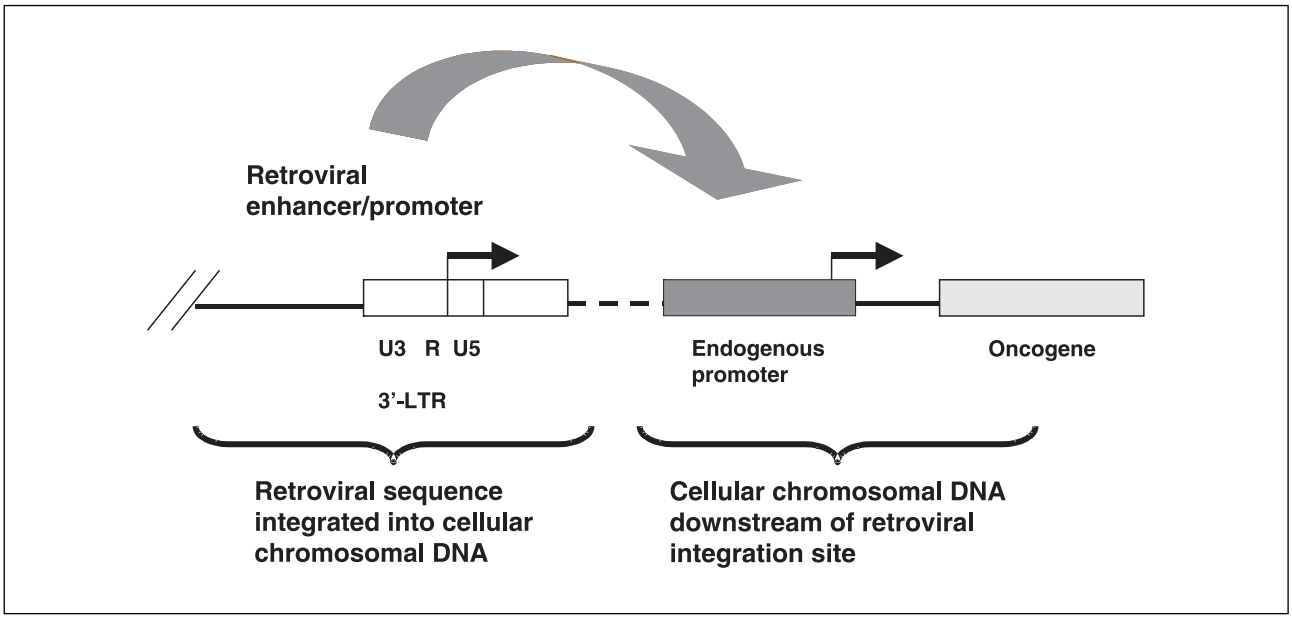

Fig. 2. Interaction between a retroviral enhancer and an endogenous oncogene promoter. A retroviral genome is integrated into the cellular chromosomal DNA. The retroviral enhancer may eventually increase the transcriptional activity of an endogenous promoter of a cellular oncogene. This type of interaction may have a long range of action.

vided by the bovine papillomavirus E5 protein, which shares a high degree of homology with the platelet-derived growth factor receptor (PDGFR), and spleen focus-forming virus (SFFV) gp55 is homologous to the erythropoietin receptor (1).

A third strategy to induce cell proliferation that is adopted by many DNA viruses consists of neutralizing cellular tumor suppressor genes (1), which have the function of limiting the cell proliferation program to a certain level (1, 14). The neutralization of tumor suppressor genes necessarily results in increased cell proliferation. A number of viral factors have been reported to target tumor suppressor genes. For instance, the SV40 large T antigen has the ability to inactivate both *p53* and retinoblastoma tumor suppressor genes (1, 13, 14). Proteins E6 and E7 of HPV neutralize *p53* and retinoblastoma tumor suppressor gene, respectively (1). Adenoviral E1B protein inhibits *p53* gene expression (1), whereas adenoviral E1A protein neutralizes the expression of retinoblastoma tumor suppressor gene (1).

Indeed, SV40 large T antigen was extensively used in the past for *in vitro* investigation of cell transformation. In addition, SV40 and most human adenovirus serotypes, with the only exception of serotypes 2 and 5, cause tumors in animal models (2, 3, 15). However, none of the adenovirus serotypes has ever been seen to cause

tumors in humans (2, 3, 15) and the possible involvement of SV40 in human malignancies remains a controversial issue (16). SV40 was detected as a contaminant of polio vaccine stocks which were administered to approximately 100 million subjects in the United States alone from 1953 to 1963 (16). The ability of SV40 to transform *in vitro* human cells and induce tumors in animal models raised a great deal of concern for its potential harm to those individuals who were inadvertently inoculated with contaminated lots of polio vaccines. However, despite the large number of individuals exposed to SV40, there is no epidemiological study that can adequately link SV40 transmission with an increased incidence of malignancies in the human population (16).

Conversely, the role of HPV, HBV and HCV in human tumor development is well established (17-30). Every year, 10 million individuals are diagnosed with various types of cancer worldwide (18). More than 15% of these newly diagnosed tumors are caused by infectious agents, such as HPV, HBV, HCV and *Helicobacter pylori* (18). HPV accounts for roughly 30% of new cancers with infectious etiology, and HBV, HCV and *H. pylori* are responsible for an additional 60% of such tumors (18). HPV is associated with the majority of cervical cancers, the second cause of cancer-related deaths among women (18). HBV and HCV are linked to the development of hepatocellular carcinoma (HCC), one of the most common lethal malignancies throughout the world (24-26, 29). Preventive vaccines have been developed against HBV (24) and, more recently, HPV (17, 18).

The aim of this review is to discuss the mechanism of HPV-, HBV- and HCV-induced carcinogenesis in humans, along with the current status of vaccine development to prevent HBV and HPV transmission.

Viral-induced HCC and development of preventive vaccines against HBV

According to the latest estimates, HCC is the third leading cause of cancer-related death in the world and its incidence is increasing. Chronic HBV and HCV infection is linked to over 70% of the reported cases of HCC (24-26, 29). Other factors that may be involved in HCC development comprise consumption of drugs and toxins (alcohol, anabolic steroids, aflatoxins, microcystin), diabetes, nonalcoholic fatty liver diseases, steatosis and monogenic disorders leading to metabolic liver diseases, such as α_1-antitrypsin deficiency and hereditary hemochromatosis (29). All these etiological agents appear to induce the malignant transformation of hepatocytes via the mechanism of chronic liver injury and regeneration, which in turn induces an increase in liver cell turnover (24-26, 29).

The clinical course of disease leading to HCC involves three stages, independent of the etiological agent: chronic hepatitis, liver cirrhosis and, finally, HCC (24-26, 29). HCC results from the alteration of at least one of the four main pathways that regulate either cell proliferation or programmed cell death (apoptosis). These four pathways comprise: p53, phospho-retinoblastoma (pRb), the β-catenin signaling system and transforming growth factor-β (TGF-β) (31, 32). Indeed, over 50% of HCCs reveal alterations of β-catenin and/or Wnt signaling systems (32). Other important components of hepatocarcinogenesis consist of increased levels of expression of growth factors, such as TGF-α, TGF-β, insulin-like growth factor (IGF)-2 and hepatocyte growth factor (HGF), and constitutive activation of their signaling systems, such as the insulin receptor substrate (IRS)-1-associated intracellular signaling pathway (29).

As would be expected, HCC is the result of effects related to chronic liver injury and regeneration. However, there is consistent experimental evidence from *in vitro* models indicating the possibility that HCV might also have the ability to promote malignant transformation of hepatocytes via direct mechanisms, mainly involving three viral proteins: HCV core (33-36), NS3 (37-39) and NS5A (40-43). These three viral proteins interact with a variety of cellular factors, which are listed in Table I. In addition, a fourth HCV protein, termed NS4B, acts as a cofactor for NS3 in the malignant transformation of hepatocytes (29).

Interestingly, the interactions among HCV core, NS3, NS5A and various cellular factors can also affect host immune responses to HCV (43-45). For instance, HCV core can bind the complement receptor C1qT of T-cells, with consequent host immunosuppression (44). NS3 has the ability to inhibit interferon response factor (IRF)-3, which is otherwise induced by interferon type I in the natural course of mounting a host immune response to viral

Table I: List of putative HCV-transforming proteins that interact with various cellular factors.

HCV protein	Cellular factor
HCV core	p53 pRb 14-3-3 LZIP hnRNP K RNA helicase DEAD box DDX3 TNF receptor Lymphotoxin β receptor
NS3	p53 H2B H4 Protein kinase A (PKA) Protein kinase C (PKC) Interferon response factor (IRF)-3
NS5A	IRFs Protein kinase R (PKR) SNAP receptor TATA box-binding protein (TBP) p53 hTAF(II)28 hTAF(II)32 Growth factor receptor-bound protein 2 adaptor protein (G2b2) Phosphatidylinositol 3-kinase (PI3K) CDK2 Forkhead (inhibition) GSK-3 (inactivation)

infection (45). Thus, the action of NS3 may also have important implications in the ability of HCV to escape immune surveillance (45). Lastly, NS5A can inhibit IRFs and induce the expression of IL-8 (43). The latter leads to the suppression of antiviral effects mediated by interferons (43). However, to date, there is still no vaccine available to prevent the transmission of HCV.

The role of HBV infection in hepatocarcinogenesis is also very complex. On the one hand, chronic liver inflammation along with constitutive liver regeneration greatly contributes to the accumulation of genetic alterations in infected hepatocytes, which may eventually lead to tumor formation and disease progression (24-26). In this respect, it is generally recognized that chronic inflammation and the development of cancer are two sides of the same coin, although their molecular links are not entirely understood (46). On the other hand, although HBV does not seem to have direct cytopathological and oncogenic effects in infected hepatocytes, certain viral components might contribute to hepatocarcinogenesis by aggravating the severity of the damage induced by host immune responses (25). For instance, the persistent expression of HBV-regulatory X protein and of the large surface protein LHBs might play a part in increasing the adverse effects of chronic liver inflammation by incessant challenge of the host immune system (47). Furthermore, X protein transactivates transcription from HBV promoters and stimulates transcription from an assortment of cellular genes, such as oncogenes, growth factors and cytokines (47). Although HBV X protein does not seem to have any direct role in establishing a transformed cell phenotype, X protein may function as a cofactor in various models of hepatocarcinogenesis (48). For example, HBV X protein was able to enhance c-myc-induced liver carcinogenesis in transgenic mice (48).

Another aspect of the biology of HBV that is under investigation is the phenomenon of insertional mutagenesis (25), which may play a role in promoting tumorigenesis (2-5). HBV has the ability to integrate its genome into the chromosomes of infected hepatocytes (25). The integration of HBV DNA occurs randomly in the target cell genome (49), but with a certain bias for cellular genes involved in cell signaling and growth control (50). In addition, the integration of the HBV genome may increase cellular chromosomal instability and therefore give rise to a variety of genetic rearrangements, such as chromosomal translocations, deletions and amplifications (51). Indeed, these events triggered by HBV-induced insertional mutagenesis were found in several human tumors (51). In most cases, inactivation of *p53* is detected in cancers associated with HBV infection, whereas HBV-related tumors exhibit quite a low rate of β-catenin mutations or deregulation (25). Such a trend is in contrast with HCC induced by other etiological agents, in which β-catenin alterations are present in more than 50% of tumors. On these grounds, one might suggest that chronic HBV infection appears to be able to trigger specific pathways for the malignant transformation of hepatocytes. This would mean that HBV might exert an additional effect on the persistent stimulation of host immune responses leading to chronic necroinflammatory hepatic disease.

According to a World Health Organization (WHO) report, more than 350 million individuals are affected by chronic HBV infection worldwide (24). Patterns of HBV transmission vary enormously among human populations. For instance, in Western countries HBV infection is usually acquired in adulthood and is less frequent than in Asia and Africa (24). Instead, in the latter two continents, HBV transmission predominantly occurs from mother to child and from child to child in household settings (24).

Effective and safe preventive vaccines against HBV transmission have been available since 1982 (24). These preventive vaccines include a mixture of recombinant HBV core and surface antigens in an aluminum-based adjuvant (52). The WHO is aiming for the global prevention of HBV infection. Up until 2005, 147 nations had implemented mass vaccinations for HBV in newborns and children, while a coalition of private and public institutions, the Global Alliance for Vaccine and Immunization (GAVI), is providing support to 74 low-income nations for immunization programs against HBV and other infectious agents.

HPV-induced cervical cancer and development of preventive vaccines

As previously mentioned, 5% of the 10 million new cases of tumors that are diagnosed worldwide on a yearly basis are related to HPV-induced cervical cancers (18). Various HPV serotypes have the ability to infect the stratified squamous epithelia and mucous membranes (53, 54). In most cases, HPV infection generates benign lesions. However, some of these lesions have the potential to become malignant (54-57).

HPV is a small nonenveloped DNA virus with a circular genome of approximately 8 kb. The HPV genome encodes two structural proteins, termed L1 and L2, which constitute the viral capsid (18). Additionally, the HPV genome encodes for other nonstructural factors that are not incorporated into the virions. These nonstructural factors are necessary for the regulation of the viral life cycle and replication.

In order to achieve effective infection, HPV must be transmitted to epithelial cells of the basal layer of the epidermis. Basal epithelial cells have stem cell-like properties and are long-lived (18). Microtrauma or erosion of the suprabasal epidermal layers is required to allow HPV to reach and then infect basal epithelial cells, where the viral genome remains in an episomal state. At this stage, HPV genes are expressed at very low levels. Both epidermal cell differentiation and concomitant migration are responsible for HPV transmission to suprabasal layers of the epidermis, where viral replication can take place. However, only low expression of HPV noncapsid genes can be observed in the early stages of epidermal cell differentiation and migration in the region of the epidermis proximal to the basal layers. With the progression of epidermal cell differentiation and migration, it becomes possible to detect the expression of capsid and noncapsid

HPV genes. This is the region of the epidermis that is distal from the basal layer. Ultimately, desquamated cells of the epidermal surface release HPV.

The hallmarks of HPV infection typically consist of aberrant epithelial morphology, papillomatosis, kollocytosis and parakeratosis (18). HPV-induced benign lesions comprise nongenital skin warts, anogenital epidermal warts, anogenital mucosal condylomata, oral and laryngeal papillomas. Long-term infection of certain HPV serotypes can cause the onset of malignant anogenital tumors, such as cancer of the cervix, vulva, vagina, penis and anus (54, 55, 57).

Cervical cancer is by far the most frequent HPV-induced tumor, and in terms of cancer-related death among women worldwide it is second only to breast cancer (57, 58). Practically all cases of cervical cancer derive from sexual HPV transmission (57-60). There are about 15 potentially oncogenic HPV serotypes. However, HPV-16 and HPV-18 are predominant in inducing cervical cancer (61). HPV-16 accounts for approximately 50% of cervical cancer cases, and HPV-18 is responsible for an additional 20% (61).

The average disease-free interval from HPV infection to the onset of cervical carcinoma is in the range of 12-15 years (58). Effective preventive vaccines against HPV infection have been developed recently by two pharmaceutical companies: GlaxoSmithKline (Cervarix™) and Merck & Co. (Gardasil®) (62-65). In these vaccines, the HPV main structural capsid protein, termed L1, is assembled to form virus-like particles (VLPs) that carry HPV immunodominant epitopes required for neutralization of the virus via host humoral immune responses (62-65). GlaxoSmithKline generates HPV VLPs with a recombinant baculovirus system and insect cells (62, 63), whereas the Merck vaccine is derived from yeast (65). The adjuvant of the GlaxoSmithKline vaccine is based on a mixture of alum and monophosphoryl lipid A, a modified lipopolysaccharide. On the other hand, the Merck vaccine uses alum alone as adjuvant. Both vaccines elicit effective host humoral immune responses and require three boosts over a period of 6 months, which are administered via intramuscular injection. The products of both companies have been designed to confer host protective immunity against HPV-16 and HPV-18, the etiological agents of 70% of the reported cases of cervical cancer (61). In addition, the Merck vaccine targets HPV-6 and HPV-11. These two HPV serotypes generate external genital warts (66) and can infect tissues of the cervix, although they are not involved in the development of cervical cancer (66).

Conclusions

The development of preventive vaccines directed against oncogenic viruses may allow for a substantial decline in cancer incidence and mortality. In this respect, vaccines are currently available for the prevention of HBV and HPV transmission, while several ongoing research programs are aimed at producing prophylactic vaccines for HCV and *H. pylori* infection. Another daunting task consists of implementing mass vaccinations for HBV and HPV infection in low-income countries.

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References

1. Drummond-Barbosa, D., DiMaio, D. *Virocrine transformation*. Biochim Biophys Acta 1997, 1332: M1-17.

2. Romano, G., Micheli, P., Pacilio, C., Giordano, A. *Latest developments in gene transfer technology: Achievements, perspectives, and controversies over therapeutic applications*. Stem Cells 2000, 18: 19-39.

3. Romano, G., Pacilio, C., Giordano, A. *Gene transfer technology in therapy: Current applications and future goals.* Stem Cells 1999, 17: 191-202.

4. Romano, G. *Gene transfer in experimental medicine*. Drug News Perspect 2003, 16: 267-76.

5. Romano, G., Claudio, P.P., Tonini, T., Giordano, A. *Human immunodeficiency type 1 (HIV-1) derived vectors: Safety considerations and controversy over therapeutic applications.* Eur J Dermatol 2003, 13: 424-9.

6. Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M. et al. *LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1*. Science 2003, 302: 415-9.

7. Cavazzana-Calvo, M., Hacein-Bey-Abina, S., de Saint Basile, G. et al. *Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease*. Science 2000, 288: 669-72.

8. McCormack, M.P., Rabbitts, T.H. *Activation of the T-cell oncogene LMO2 after gene therapy for X-linked severe combined immunodeficiency*. New Engl J Med 2004, 350: 913-22.

9. Knecht, H., Berger, C., Al-Homsi, A.S. et al. *Epstein-Barr virus oncogenesis*. Crit Rev Oncol Hematol 1997, 26: 117-35.

10. Gabusi, E., Lattes, C., Fiorentino, M. et al. *Expression of Epstein-Barr virus-encoded RNA and biological markers in Italian nasopharyngeal carcinomas*. J Exp Clin Cancer Res 2001, 20: 371-6.

11. Yang, J., Deng, X., Deng, L. et al. *Telomerase activation by Epstein-Barr virus latent membrane protein 1 is associated with c-Myc expression in human nasopharyngeal epithelial cells.* J Exp Clin Cancer Res 2004, 23: 495-506.

12. Yoshiwara, E., Koriyama, C., Akiba, S. et al. *Epstein-Barr virus-associated gastric carcinoma in Lima, Peru*. J Exp Clin Cancer Res 2005, 24: 49-54.

13. Wang, J.Y., Del Valle, L., Peruzzi, F. et al. *Polyomaviruses and cancer — Interplay between viral proteins and signal transduction pathways*. J Exp Clin Cancer Res 2004, 23: 373-83.

14. Gan, D.D., Macaluso, M., Cinti, C. et al. *How does a normal human cell become a cancer cell?* J Exp Clin Cancer Res 2003, 22: 509-16.

15. Romano, G. *The controversial role of adenoviral-derived vectors in gene therapy programs: Where do we stand?* Drug News Perspect 2006, 19: 99-106.

16. Poulin, D.L., DeCaprio, J.A. *Is there a role for SV40 in human cancer?* J Clin Oncol 2006, 24: 4356-65.

17. Schiller, J.T., Lowy, D.R. *Prospects for cervical cancer prevention by human papillomavirus vaccination*. Cancer Res 2006, 66: 10229-32.

18. Lowy, D.R., Schiller, J.T. *Prophylactic human papillomavirus vaccines*. J Clin Invest 2006, 116: 1167-73.

18. Cianfriglia, F., Di Gregorio, D.A., Cianfriglia, C. et al. *Incidence of human papillomavirus infection in oral leukoplakia.* J Exp Clin Cancer Res 2006, 25: 21-8.

19. Venuti, A., Badaracco, G., Rizzo, C. et al. *Presence of HPV in head and neck tumors: High prevalence in tonsillar localization.* J Exp Clin Cancer Res 2004, 23: 561-6.

20. Lazaris, A.C., Chatzigianni, E.B., Xidias, G. et al. *Tissue evaluation of immune markers in endometrial and cervical carcinomas*. J Exp Clin Cancer Res 2004, 23: 269-75.

21. Vocaturo, A., Marandino, E., Celata, E. et al. *Colourimetric signal amplification of in situ hybridization assay for human papillomavirus DNA detection in cytological samples*. J Exp Clin Cancer Res 2002, 21: 239-46.

22. Indinnimeo, M., Cicchini, C., Stazi, A. et al. *Human papillomavirus infection and p53 nuclear overexpression in anal canal carcinoma*. J Exp Clin Cancer Res 1999, 18: 47-52.

23. Henning, E.M., Di Lonardo, A., Venuti, A. et al. *HPV16 in multiple neoplastic lesions in women with CIN III*. J Exp Clin Cancer Res 1999, 18: 369-77.

24. Lavanchy, D. *Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention*. J Clin Virol 2005, Suppl. 1: S1-3.

25. Cougot, D., Neuveut, C., Buendia, M.A. *HBV-induced carcinogenesis*. J Clin Virol 2005, Suppl. 1: S75-8.

26. Villeneuve, J.P. *The natural history of chronic hepatitis B infection*. J Clin Virol 2005, Suppl. 1: S139-42.

27. Matsuzaki, Y., Sato, M., Saito, Y. et al. *The role of previous infection of hepatitis B virus in HBs antigen negative and anti-HCV negative Japanese patients with hepatocellular carcinoma: Etiological and molecular biological study*. J Exp Clin Cancer Res 1999, 18: 379-89.

28. Vasudevan, D.M., Vijayakumar, T. *Viruses in human oral cancers*. J Exp Clin Cancer Res 1998, 17: 27-31.

29. Levrero, M. *Viral hepatitis and liver cancer: The case of hepatitis C*. Oncogene 2006, 25: 3834-47.

30. Su, Y.H., Lu, S.L., Gu, Y.H. et al. *In situ RT-PCR detection of hepatitis C virus genotypes in Chinese patients with hepatocellular carcinoma*. J Exp Clin Cancer Res 2002, 21: 591-8.

31. Moradpour, D., Blum, H.E. *Pathogenesis of hepatocellular carcinoma*. Eur J Gastroenterol Hepatol 2005, 17: 477-83.

32. Ozturk, M. *Genetic aspects of hepatocellular carcinogenesis*. Semin Liver Dis 1999, 19: 235-42.

33. Alisi, A., Giambartolomei, S., Cupelli, F. et al. *Physical and functional interaction between HCV core protein and different p73 isoforms*. Oncogene 2003, 22: 2573-80.

34, Cho, J., Baek, W., Yang, S. et al. *HCV core protein modulates Rb pathway through pRb down-regulation and E2F-1 up-regulation*. Biochim Biophys Acta 2001, 1538: 59-66.

35. Lu, W., Ou, J.H. *Phosphorylation of hepatitis C core protein by protein kinase A and protein kinase C*. Virology 2002, 300: 20-30.

36. Ray, R.B., Steele, R., Meyer, K., Ray, R. *Transcriptional repression of p53 promoter by hepatitis C virus core protein*. J Biol Chem 1997, 272: 10983-6.

37. Ishido, S., Muramatsu, S., Fujita, T. et al. *Wild-type, but not mutant type, p53 enhances nuclear accumulation of the NS3 protein of hepatitis C virus*. Biochem Biophys Res Commun 1997, 230: 431-6.

38. Ishido, S., Hotta, H. *Complex formulation of the nonstructural protein 3 of hepatitis C virus with the p53 tumor suppressor*. FEBS Lett 1998, 438: 258-62.

39. Sakamuro, D., Furukawa, T., Takegami, T. *Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells*. J Virol 1995, 69: 3893-6.

40. Tu, H., Gao, L., Shi, S.T. et al. *Hepatitis C virus RNA polymerase and NS5A complex with a SNARE-like protein*. Virology 1999, 263: 30-41.

41. Chung, K.M., Lee, J., Kim, J.E. et al. *Nonstructural protein 5A of hepatitis C virus inhibits the function of karyopherin beta3*. J Virol 2000, 74: 5233-41.

42. Gale, M.J., Korth, M.J., Tang, N.M. et al. *Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein*. Virology 1997, 230: 217-27.

43. Polyak, S.J., Khabar, K.S., Paschal, D.M. et al. *Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response*. J Virol 2001, 75: 6095-106.

44. Kittlesen, D.J., Chianese-Bullock, K.A., Yao, Z.Q. et al. *Interaction between complement receptor gC1qR and hepatitis C virus core protein inhibits T-lymphocyte proliferation*. J Clin Invest 2000, 106: 1239-49.

45. Foy, E., Li, K., Wang, C. et al. *Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease*. Science 2003, 300: 1145-8.

46. Clevers, H. *At the crossroads of inflammation and cancer*. Cell 2004, 118: 671-4.

47. Murakami, S. *Hepatitis BH virus X protein: Structure, function and biology*. Intervirology 1999, 42: 81-99.

48. Terradillos, O., Billet, O., Renard, C.A. et al. *The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice*. Oncogene 1997: 14: 395-404.

49. Matsubara, K., Tokino, T. *Integration of hepatitis B virus DNA and its implications for hepatocarcinogenesis*. Mol Biol Med 1990, 7: 243-60.

50. Paterlini-Brechot, P., Saigo, K., Murakami, Y. et al. *Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene*. Oncogene 2003, 22: 3911-6.

51. Buendia, M.A. *Genetics of hepatocellular carcinoma*. Sem Cancer Biol 2000, 10: 185-200.

52. Iglesias, E., Franch, O., Carrazana, Y. et al. *Influence of aluminum-based adjuvant on the immune response to multiantigenic formulation*. Viral Immunol 2006, 19: 712-21.

53. Bosch, F.X., Schiffman, M., Solomon, D. *Future directions in epidemiology and preventive research on human papillomaviruses and cancer*. J Natl Cancer Inst Monogr 2003, 31: 131.

54. Zur Hausen, H. *Papillomaviruses and cancer: From basic studies to clinical application.* Nat Rev Cancer 2002, 2: 342-50.

55. Frisch, M. *On the etiology of anal squamous carcinoma*. Dan Med Bull 2002, 49: 194-209.

56. Gillison, M.L., Lowy, D.R. *A causal role for human papillomavirus in head and neck cancer.* Lancet 2004, 363: 1488-9.

57. Yang, B.H., Bray, F.J., Parkin, D.M. et al. *Cervical cancer as a priority for prevention in different world regions: An evaluation using years of life lost.* Int J Cancer 2004, 109: 418-24.

58. Snijders, P.J., Steenbergen, R.D., Heideman, D.A., Meijer, C.J. *HPV-mediated cervical carcinogenesis: Concepts and clinical implications.* J Pathol 2006, 208: 152-64.

59. Bosch, F.X., Lorincz, A., Munoz, N. et al. *The causal relation between human papillomavirus and cervical cancer.* J Clin Pathol 2002, 55: 244-65.

60. Wright, T.C., Cox, J.T., Massad, L.S. et al. *2001 consensus guidelines for the management of women with cervical cytology abnormalities*. JAMA – J Am Med Assoc 2002, 287: 2120-9.

61. Munoz, N., Bosch, F.X., Castellsague, X. et al. *Against which papillomavirus types shall we vaccinate and screen?* Int J Cancer 2004, 111: 278-85.

62. Harper, D.M., Franco, E.L., Wheeler, C.M. et al. *Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: Follow-up from a randomized control trial*. Lancet 2006, 367: 1247-55.

63. Harper, D.M., Franco, E.L., Wheeler, C.M. et al. *Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: A randomized controlled trial.* Lancet 2004, 364: 1757-65.

64. Koutsky, L.A., Ault, K.A., Wheeler, C.M. et al. *A controlled trial of a human papillomavirus type 16 vaccine.* New Engl J Med 2002, 347: 1645-51.

65. Villa, L.L., Costa, R.L., Petta, C.A. et al. *Prophylactic quadrivalent human papillomavirus (types 6, 11, 16 and 18) L1 virus-like particle vaccine in young women: A randomized double-blind placebo-controlled multicentre phase II efficacy trial.* Lancet Oncol 2005, 271-8.

66. Greer, C.E., Wheeler, C.M., Ladner, M.B. et al. *Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts*. J Clin Microbiol 1995, 33: 2058-63.