American Journal of Pathology, Vol. 145, No. 5, November 1994
Copyright © American Society for Investigative Pathology

Short Communication

Hepatitis B Virus Infection Associated with Hematopoietic Tumors

Eithan Galun,* Y. Ilan,* N. Livni,† M. Ketzinel,* O. Nahor,* G. Pizov,† A. Nagler,‡ A. Eid,§ A. Rivkind,§ M. Laster,* N. Ron,† H.E. Blum,‖ and D. Shouval*

From the Liver Unit, Division of Medicine, Department of Pathology,† Department of Bone Marrow Transplantation,‡ and Department of Surgery,§ Hadassah University Hospital, Ein-Kerem, Jerusalem, Israel; and Department of Medicine,‖ University Hospital Zürich, Zürich, Switzerland*

Hepatitis B virus (HBV) infection and replication have been linked to the development of hepatocellular carcinoma. Bone marrow-derived cells, as well as mesenchymal and epithelial cells, were recently shown to support HBV replication. We hypothesize that the mechanism that links HBV infection and liver tumors might also promote tumor development in tissues permissive for HBV replication. Between 1980 and 1993 we retrospectively identified 22 patients who were hepatitis B surface antigen (HBsAg) carriers and had extrahepatic malignancies. These patients had 25 tumors, of which 22 were bone marrow derived. HBsAg was detected by immunohistochemistry in bone marrow cells of leukemia patient and of 3 of 10 lymphoma patients. In addition, in 4 of 10 patients with lymphoma, including 2 patients in which HBsAg stained bone marrow cells, HBsAg was also detected in the endothelial cells of blood vessels of the tumor tissue. These results suggest that the identification of an HBV gene product in endothelial cells might point to a role of HBV infection in the development of certain hematopoietic tumors, possibly through activation of cytokines or growth factors, which may eventually lead to bone marrow cell proliferation. (Am J Pathol 1994, 145:1001–1007)

Hepatitis B virus (HBV) infection is closely associated with the development of hepatocellular carcinoma (HCC).[1] The exact pathogenesis leading to chronic HBV infection and HCC is only partially understood.[2] Different molecular mechanisms contributing to hepatocarcinogenesis have been proposed, including: 1) HBV-DNA integration into genome of hepatocytes,[3] causing genomic instability or interference with cell cycle regulation through HBV-DNA integration into the cyclin A gene;[4] 2) accumulation of HBV envelope proteins in hepatocytes, leading to hepatocellular injury, inflammation, regenerative hyperplasia, and progression to HCC, as shown in a transgenic mice model;[5] 3) a direct effect of HBV-encoded proteins like the HBV-X gene product, which stimulates gene expression by *trans*-activation,[6] as recently reported for protein kinase C, which plays a key role in cellular signal transduction.[7] The relevance and significance of each of these molecular processes for the development HCC is still unknown.

Recent advances in the molecular biology of HBV have identified viral DNA, RNA, or proteins not only in hepatocytes, but also in a number of extrahepatic sites, such as lymphoblastoid cells, lymph nodes, bile ducts, and vascular elements in the liver.[8–11] In addition, we have recently shown that transfected HBV-DNA as well as other members of the hepadnaviruses family, such as the duck HBV, replicate in nonliver epithelial and mesenchymal cells.[12] Such extrahepatic sites may play an important role in the natural course of HBV infection.

Tropism of hepadnaviruses, including HBV, to hematopoietic cells,[13] nonliver epithelial cells,[14] and mesenchymal cells[15] and the permissiveness of these extrahepatic cells for viral replication might also promote tumor development in these cells and tis-

---

Supported in part by the L. Naftali Foundation and the Richard Molin Memorial Foundation for Cancer Research.

Accepted for publication August 12, 1994.

Address reprint requests to Dr. Eithan Galun, Liver Unit, Division of Medicine, Hadassah University Hospital, Ein-Kerem, P.O. Box 12000 Jerusalem 91120, Israel.

sues. To address this hypothesis, we have analyzed nonliver-related tumors for the presence of HBV infection.

Materials and Methods

Patients

Through the institutional computerized registry system, we have searched retrospectively for patients with nonliver-related primary tumors hospitalized between the years 1980 and 1993 who were tested for HBV infection. Forty-four patients with HBV infection markers were identified, of whom 22 were excluded from the study because their HBV status was unknown before the tumor diagnosis. Table 1 shows the oncological diagnosis of the 22 patients of the study group.

Immunohistochemistry

Paraffin-embedded tissues were cut into 4- to 6-μ thick sections, deparaffinized in xylene and alcohol, and placed for 15 minutes in alcohol-H_2O_2 for blocking endogenous peroxidase. Slides were thoroughly washed with tap water and transferred to phosphate-buffered saline (PBS). Sections were then treated with bovine serum albumin to prevent background staining and incubated for 1 hour with a primary murine antihepatitis B surface antibody (Zymed Laboratories, Inc., San Francisco, CA) at room temperature in a humidified chamber. Slides were rinsed with PBS for 3 minutes and incubated with the biotinylated-linked goat anti-mouse antibody for 30 minutes and with the labeling reagent, peroxidase-conjugated streptavidin, for 30 minutes (Biogenex StrAvigen, San Ramon, CA). After rinsing, the peroxidase label was demonstrated using 3-amino-9-ethylcarbazol (AEC) for 15 minutes and counterstained with Mayer hematoxylin. AEC produced a red product that is soluble in alcohol and is used with an aqueous mounting medium (Kaiser's glycerol gelatin). A negative control was run using the same technique but omitting the primary antibody and adding the streptavidin-biotin complex. For each tumor in the study group stained for hepatitis B surface antigen (HBsAg), a matched tumor from a patient without HBV markers was stained as a negative control. Endothelial cells were stained for factor VIII with rabbit anti-human factor VIII-related antigen (Dako, Glostrup, Denmark) as a primary antibody, followed by incubation with peroxidase-conjugated goat anti-rabbit (Jackson Immunoresearch, West Grove, PA) second antibody incubation.

Analysis of HBV-Related Antigens in the Serum

The sera of patients was stored at −20 C then thawed and analyzed for HBsAg with an enzyme-linked immunoassay kit (Ausria; Abbott Laboratories, Abbott Park, IL) and HBV c/e antigen (HBeAg) with a radioimmunoassay (Abbott Laboratories).

HBV-DNA Analysis

In Tissue

Presence of HBV-DNA sequences in tumor tissue was assessed by methods previously described.[16–19] Briefly, formalin-fixed, paraffin-embedded tissue sections were cut from the original paraffin block with a razor blade and approximately 12 mg tissue was inserted into 1.5 ml of microfuge tube. The paraffin sections were extracted twice with xylene for 5 to 10 minutes at 55 C to remove paraffin, followed by two washes with 100% ethanol to remove xylene. The tissue was digested for 2 hours at 55 C in 500 μl STE (10 mmol/L Tris Cl, pH 7.5, 10 mmol/L NaCl, and 1 mmol/L EDTA) containing 500 μg proteinase K (Boehringer Mannheim, Mannheim, Germany) per ml and 1% sodium dodecyl sulfate. After phenol extraction and ethanol precipitation, the DNA species were fractionated through a 1.25% agarose gel, transferred to a Nytran membrane (Schleicher & Schuell, Dassel, Germany), and hybridized with a full-length HBV-DNA probe ^{32}P labeled by random priming. Prehybridization and hybridization were conducted according to Sambrook et al.[20]

For the preparation of genomic DNA from 6×10^7 peripheral blood nucleated cells, DNA was extracted using a salting out procedure,[21] precipitated, digested, and fractionated as described for the DNA extracted from the paraffin blocks.

Polymerase chain reaction (PCR) was performed on extracted DNA in a 50 μl reaction mixture containing (final concentration) 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 2.5 mmol/L $MgCl_2$, 2.5 mmol/L of each dNTP, 2.5 U Taq polymerase (Promega, Madison, WI), and 50 ng of each primer. PCR was performed with HBV core and envelope sequence primers. Primers (nucleotide number starts at *Eco*RI site): core sense (map position 1776 to 1804 5′-GGAGGCTGTAGGCATAAATTGGTCTGCGC-3′), core antisense (map position 2439 to 2401 5′-CCCGAGATTGAGATCTTCTGCGACGCGGCGATTGAGACC-3′), envelope sense (map position 2819 to 2847 5′-TGGAGCTCACCATATTCTTGGGAACAAGA-3′), envelope antisense (map position 850 to 823 5′-GTA-

AGCTTAGGGTTTAAATGTATACCCA-3'). The reaction was conducted in 35 cycles of 94 C for 1 minute, 55 C for 1 minute, and 72 C for 1 minute, with a 10-minute extension step at 72 C at the end. Ten microliters of the products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

In Serum

HBV-DNA sequences in the serum were detected by dot blot hybridization[22] and PCR performed on DNA extracted from 200 to 500 µl of serum using the core primers in a similar method as described for PCR on DNA extracted from paraffin blocks.

Results

Twenty-two patients with 25 tumors were studied. Nine patients had leukemia; four of these had leukemic cells in the peripheral blood during the study period. Ten patients had lymphoma, seven non-Hodgkin's lymphoma, one Hodgkin's lymphoma, one Burkitts' lymphoma, one immunoblastic lymphoma, one had malignant histiocytosis, and one had Castelman's disease (Table 1). Two patients had nonhematopoietic tumors (gallbladder carcinoma and Kaposi's sarcoma in one and vocal cord polyp in the other). One patient with lymphoma subsequently developed leukemia several years later.

All patients were HBsAg positive before and at the time of tumor diagnosis. Serum was available for analysis of viral replication in 19 patients of whom 10 had evidence for active viral replication, as detected by HBeAg and/or HBV-DNA sequences as assessed by dot blot and confirmed by PCR. HBV-DNA as measured by dot blot hybridization was not identified in leukemic cells or solid tumors in 4 and 10 patients, respectively. However, HBsAg was detected by immunohistochemistry in endothelial cells within tumor

Table 1. *HBV Infection In Nonliver-Related Tumors*

Patient No.	Tumor Histology	Serum HBV Infection Markers				HBsAg staining of Tumor
		HBsAg	HBeAg	HBV DNA Dot Blot	HBV DNA PCR	
1	Burkitts lymphoma	+	+	+	+	Lymph node: negative
2	Acute promyelocytic leukemia	+	+	+	+	Bone marrow and peripheral blood cells: negative Megakaryocytes are stained false positive
3	Malignant histiocytosis	+	+	+	–	Lymph node: NA
4	Non-Hodgkin's lymphoma	+	–	–	–	Lymph node: negative
5	Non-Hodgkin's lymphoma	+	–	+	+	Lymph node: positive in lymphocytes Bone marrow: positive in lymphoid lineage cells
6	Non-Hodgkin's lymphoma	+	–	+	NA	Lymph node: negative
	Kaposi's sarcoma					Blood vessels: positive in endothelial cells
	Gallbladder carcinoma					Carcinoma epithelial cells are stained false positive
	Chronic lymphatic leukemia					Bone marrow: negative
7	Erythroleukemia	+	NA	NA	NA	Bone marrow: negative
8	Acute myeloid leukemia	+	–	–	–	Bone marrow: negative
9	Castleman's disease	+	+	–	–	Lymph node: negative
10	Non-Hodgkin's lymphoma	+	NA	NA	NA	Tonsil: negative
11	Immunoblastic lymphoma	+	–	+	–	Bone marrow: positive in lymphoid lineage cells Lymph node: positive in endothelial cells Brain metastasis: positive in endothelial cells
12	Non-Hodgkin's lymphoma	+	–	–	–	Bone marrow: positive in lymphoid lineage cells
13	Non-Hodgkin's lymphoma	+	+	+	+	Tonsil: negative Bone marrow: negative
14	Vocal cord polyp	+	–	–	NA	Polyp: NA
15	Acute lymphocytic leukemia	+	–	+	+	Bone marrow: NA
16	Acute myeloid leukemia	+	NA	NA	NA	Bone marrow: NA
17	Hodgkin's lymphoma	+	–	–	–	Lymph node: positive in endothelial cells
18	Acute myeloid leukemia	+	–	–	–	Bone marrow: positive in lymphocytes
19	Acute myeloid leukemia	+	NA	NA	NA	Bone marrow: NA
20	Polycytemia vera	+	–	–	–	Bone marrow or peripheral blood: NA
21	Non-Hodgkin's lymphoma	+	–	–	–	Spleen: positive in endothelial cells
22	Chronic lymphatic leukemia	+	+	+	NA	Bone marrow: NA

NA, not available.

blood vessels in 4 of 10 patients with lymphoma as shown in Figure 1. The phenotype of endothelial cells was confirmed through factor VIII staining. In HBsAg-positive endothelial cells, immunostaining was scattered within the cytosol near the nucleus. Furthermore, HBsAg was also detected in the bone marrow cells of 1 of 5 patients with leukemia and in lymphoma tumor cells in 2 of 10 samples tested. By applying PCR for HBV-DNA we were not able to show viral sequences in any of these cases, which were exposed in most cases to picric acid or mercuric chloride-containing fixatives.

Discussion

Hematopoietic cells that are permissive for HBV replication are probably the major extrahepatic site for hepatitis B virion production.[8,23] The bone marrow is therefore considered as the main reservoir for HBV outside the liver and may be responsible for reinfection of engrafted livers in patients with HBV infection who undergo liver transplantation.[24,25] HBV transcripts and gene products have previously been identified in bone marrow cells and endothelial cells inside the liver and in extrahepatic blood vessels.[10,26] HBV infection has also been found to be more prevalent in patients with lymphoma and leukemia. Wands et al[27] studied 85 patients with hematological malignancies and found that the carrier rates for HBsAg and anti-HBsAg among these patients were much higher than in the general population. These results were recently confirmed by a study from Japan.[28] In addition, HBV sequences were found in bone marrow cells of children who were HBsAg carriers before the diagnosis of acute leukemia.[29] This study further confirms the above reports, because in our retrospective

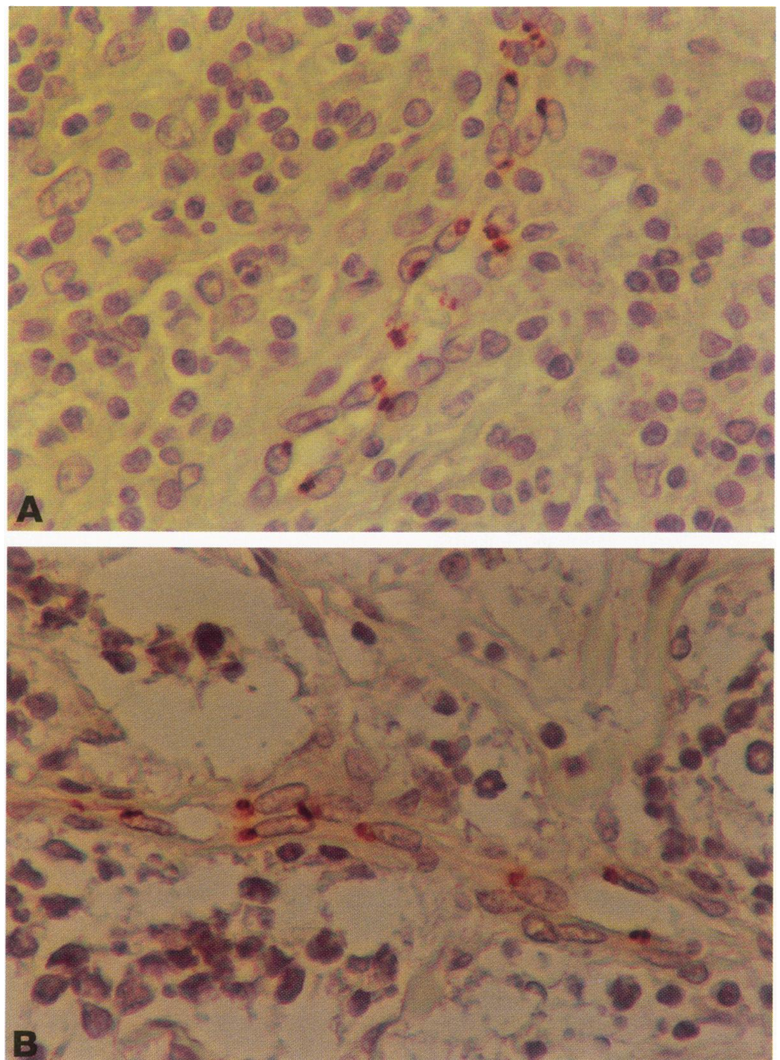

Figure 1. *Immunohistochemical detection of HBsAg.* a: *Brain metastasis from patient 11 with immunoblastic lymphoma, HBsAg is stained in red (×200).* b: *Spleen from patient 21 with non-Hodgkin's lymphoma (×200).*

search for patients with extrahepatic tumors and HBV infection, we identified 22 patients of whom 90% had hematopoietic malignancies in association with an HBsAg carrier state. HBsAg was identified in endothelial cells of 4 of 10 patients, as well as in lymphoid lineage bone marrow cells in 1 leukemic and 2 lymphoma patients. HBV-DNA was not detected in the biopsy material used for extraction by applying dot blot hybridization technique and PCR for core and envelope sequences. However, the presence of low level free cytosolic or integrated forms of viral DNA in the extrahepatic sites tested has not been excluded in view of the small samples size available, possible PCR inhibitors, and DNA strand nicking in tissue fixative chemicals.[30]

The association between HBV infection and HCC has been known for more than two decades.[31] The detection of HBV gene products in endothelial cells of hematopoietic tumors may suggest that HBV has a role in hematopoietic tumorogenesis, regardless of its role in hepatocarcinogenesis as we have previously suggested.[32] Several arguments may be used to support such an hypothesis. HBV-DNA sequences were previously detected in vascular elements in livers of HBV-infected patients, and furthermore, free HBV-DNA sequences were detected in endothelial cells of Kaposi's sarcoma from a patient chronically infected with HBV.[33]

Vascular endothelium has been shown to elaborate growth factors that participate in normal hematopoiesis.[34,35] The production of hematopoietic growth factors by vascular endothelial cells is not constitutively produced in significant quantities but could be induced by certain viruses.[36] The ability of cancer cells to respond to growth factors has become a central concept linking oncogenes to growth factors and tumor propagation.[37] Transformation of cells by Moloney, Harvey, or Kirsten viruses involves the release into culture medium of transforming growth factor-α (TGF-α), TGF-β, and platelet-derived growth factor, which leads to progression of tumor cells by autocrine[38] or paracrine effects. TGF-α elicits its potent mitogenic effect through the interaction with epidermal growth factor receptor (EGF-R).[39] In a similar way it has recently been shown that the HBV-X gene product (pX) increases the expression of EGF-R gene product.[40] pX has been identified in the serum of patients with HBV and HCC and it has been suggested that it may play a role in promoting tumor growth possibly via a paracrine effect.[41]

Taking all this data together, it might be possible that HBV infection of endothelial cells may serve as a trigger for expression, production, or release of hematopoietic tumor growth factors, which will stimulate cell proliferation. Such a mechanism has previously been suggested for other viruses expressing *trans*-activating proteins such as HTLV-1, which is recognized as an etiological agent for T cell leukemia/lymphoma.[42] The HTLV-1-associated Tax *trans*-activating protein has an homology with the HBV X protein and therefore a common mechanism may exist for the two viruses.

In conclusion, the preliminary data described in this study suggests that HBV infection may play a role in hematopoietic malignancies. This hypothesis is now being evaluated *in vitro* in transfection experiments of endothelial cell lines with HBV constructs, assessing the effect of the virus on cellular gene products that might enhance hematopoietic tumor growth.

Acknowledgments

We thank Prof. L. Blendies for reviewing the manuscript.

References

1. Beasley RP, Hwang LY, Lin CC, Chien CS: Hepatocellular carcinoma and hepatitis B virus: a retrospective study of 22707 men in Taiwan. Lancet 1981, 2:1129–1132
2. Sherker AH, Marion PL: Hepadnaviruses and hepatocellular carcinoma. Annu Rev Microbiol 1991, 45:475–508
3. Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC: Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. N Engl J Med 1981, 305: 1067–1073
4. Wang J, Chenivesse X, Henglein B, Brechot C: Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature 1990, 343:555–557
5. Chisari FV, Pinkert CA, Milich DR, Filippi P, McLachlan A, Palmiter RD, Brinster RL: A transgenic mouse model of the chronic hepatitis B surface antigen carrier state. Science 1985, 230:1157–1160
6. Kim C-M, Koike K, Saito I, Miyamura T, Jay G: HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991, 351:317–320
7. Kekule AS, Lauer V, Weiss L, Luber B, Hofschneider PH: Hepatitis B virus transactivator HBx uses a tumor promoter signalling pathway. Nature 1993, 361:742–745
8. Romet-Lemonne J-L, McLane MF, Elfassi E, Haseltine WA, Azocar J, Essex M: Hepatitis B virus infection in cultured human lymphoblastoid cells. Science 1983, 221:667–669
9. Yoffe B, Burns DK, Bhatt HS, Combes B: Extrahepatic hepatitis B virus sequences in patients with acute hepatitis B infection. Hepatology 1990, 12:187–192
10. Blum HE, Stowring L, Figus A, Montgomery CK,

Haase AT, Vyas GN: Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium, and vascular elements by *in situ* hybridization. Proc Natl Acad Sci USA 1983, 80:6685–6688
11. Zeldis JB, Mugishima H, Steinberg HN, Nir E, Gale PR. In vitro hepatitis B virus infection of human bone marrow cells. J Clin Invest 1986, 78:411–417
12. Galun E, Offensperger W-B, von Weizsäcker F, Offensperger S, Wands JR, Blum HE: Human non-hepatocytes support hepadnaviral replication and virion production. J Gen Virol 1992, 73:173–178
13. Sing GK, Prior S, Fernan A, Cooksley G: Hepatitis B virus differentially suppresses myelopoiesis and displays tropism for immature hematopoietic cells. J Virol 1993, 67:3454–3460
14. Walter E, Teubner K, Blum HE, Offensperger W-B, Offensperger S, Gerok W: Duck hepatitis B virus infection of non-hepatocytes. Liver 1991, 11:53–62
15. Korba BE, Brown TL, Wells FV, Baldwin B, Cote PJ, Steinberg H, Tennant BC, Gerin JL: Natural history of experimental woodchuck hepatitis virus infection: molecular virologic features of the pancreas, kidney, ovary, and testis. J Virol 1990, 64:4499–4506
16. von Weizsäcker F, Labeit S, Koch HK, Oehlert W, Gerok W, Blum HE: A simple and rapid method for the detection of RNA in formalin-fixed paraffin-embedded tissues by PCR amplification. Biochem Biophys Res Commun 1991, 174:176–180
17. Dubeau L, Chandler LA, Gralow JR, Nichols PW, Jones PA: Southern blot analysis of DNA extracted from formalin-fixed pathology specimens. Cancer Res 1986, 46:2964–2969
18. Yasui H, Hino O, Ohtake K, Machinami R, Kitagawa T: Clonal growth of hepatitis B virus-integrated hepatocytes in cirrhotic liver nodules. Cancer Res 1992, 52: 6810–6814
19. Jackson DP, Lewis FA, Taylor GR, Boylston AW, Quirke P: Tissue extraction of DNA and RNA and analysis by the polymerase chain reaction. J Clin Pathol 1990, 43: 499–504
20. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, ed 2. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1989
21. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988, 16:1215
22. Walter E, Blum HE, Offensperger W-B, Zeschnigk C, Offensperger S, Gerok W: Spot-blot hybridization assay for the detection of hepatitis B virus DNA in serum: factors determining its sensitivity and specificity. Hepatology 1987, 7:557–562
23. Muller C, Bergmann KF, Gerin JL, Korba BE: Production of hepatitis B virus by stably transfected monocytic cell line U-937: a model for extrahepatic hepatitis B virus replication. J Infect Dis 1992, 165:929–933
24. Missale G, Brems JJ, Takiff H, Pockros PJ, Chisari FV: Human leukocyte antigen I-independent pathways may contribute to hepatitis B virus-induced liver disease after liver transplantation. Hepatology 1993, 18: 491–496
25. Mason AL, Wick M, White HM, Benner KG, Lee RG, Regenstein F, Riely CA, Bain VG, Campbell, Perrillo RP: Increased hepatocyte expression of hepatitis B virus transcription in patients with features of fibrosing cholestatic hepatitis. Gastroenterology 1993, 105:237–244
26. Mason AL, Wick M, Swanson P, Lie J, Adams E, Hebert B, Perrillo RP: Detection of hepatitis B virus (HBV) replication in the extrahepatic tissues of patients with HBV-related syndromes (Abstract). Hepatology 1993, 18:240A
27. Wands JR, Chura CM, Roll FJ, Maddrey WC: Serial studies of hepatitis associated antigen and antibody in patients receiving anti-tumor chemotherapy for myeloproliferative and lymphoproliferative disorders. Gastroenterology 1975, 68:105–112
28. Olantunji PO, Okpala IE, Sorunmu MA: Hepatitis B surface antigenaemia in patients with malignant lymphoproliferative disorders. Tokai J Exp Clin Med 1991, 16: 171–173
29. Pontisso P, Locasciulli A, Schiavon E, Cattoretti G, Sciro R, Stenico D, Alberti A: Detection of hepatitis B virus sequences in bone marrow of children with leukemia. Cancer 1987, 59:292–296
30. Lampertico P, Malter JS, Colombo M, Gerber MA. Detection of hepatitis B virus DNA in formalin-fixed, paraffin-embedded liver tissue by the polymerase chain reaction. Am J Pathol 1990, 137:253–258
31. Okuda K: Hepatocellular carcinoma: recent progress. Hepatology 1992, 15:948–963
32. Ras E, Michaeli J, Rosenmann E, Popovtzer MM, Polliack A, Shouval D: Excessive hepatitis-B surface antigen production after corticosteroids and the development of immunoblastic lymphoma. Leukemia Lymphoma 1993, 10:241–244
33. Siddiqui A: Hepatitis B virus DNA in Kaposi sarcoma. Proc Natl Acad Sci USA 1983, 80:4861–4864
34. Islam A, Glomski C, Henderson ES: Endothelial cells and hematopoiesis: a light microscopic study of fetal, normal, and pathologic human bone marrow in plastic-embedded sections. Anat Rec 1992, 233:440–452
35. Brown TJ, Liu J, Brashem-Stein C, Shoyab M: Regulation of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor expression by oncostatin M. Blood 1993, 82:33–37
36. Bagby GC, Heinrich MC Jr: Vascular endothelial cells and hematopoiesis: regulation of gene expression in human vascular endothelial cells. Hematol Pathol 1991, 5:93–99
37. Sporn MB, Roberts AB: Autocrine growth factors and cancer. Nature 1985, 313:745–747
38. Ziober BL, Willson JKV, Hymphrey LE, Childress-Fileds K, Brattain MG: Autocrine transforming growth factor-α is associated with progression of transformation properties in human colon cancer cells. J Biol Chem 1993, 268:691–698

39. Massague J: Epidermal growth factor-like transforming growth factor. II. Interaction with epidermal growth factor receptors in human placenta membranes and A431 cells. J Biol Chem 1983, 258:13614–13620
40. Menzo S, Clementi M, Alfani E, Bagnarelli P, Iacovacci S, Manzin A, Dandri M, Natoli G, Levrero M, Carloni G: Trans-activation of epidermal growth factor receptor gene by the hepatitis B virus X-gene product. Virology 1993, 196:878–882
41. Zhu M, London T, Duan L-X, Feitelson MA: The value of hepatitis B x antigen as a prognostic marker in the development of hepatocellular carcinoma. Int J Cancer 1993, 55:571–576
42. Smith MR, Greene WC: Molecular biology of the type I human T-cell leukemia virus (HTLV-I) and adult T-cell leukemia. J Clin Invest 1991, 87:761–766