

Expression of Transforming Growth Factor beta-1 in Chronic Hepatitis and Hepatocellular Carcinoma Associated with Hepatitis C Virus Infection

Hyung Gun Kim, M.D.,* Young-Hwa Chung, M.D., Byung-Cheol Song, M.D.
JeongA. Kim, B.A., Soo Hyun Yang, M.D., Yung Sang Lee, M.D. and
Dong Jin Suh, M.D.

*Department of Internal Medicine, University of Ulsan College of Medicine,
Asan Medical Center, HanLim University, *Seoul, Korea.*

Background : *Transforming growth factor beta-1 (TGF 1) has been suggested to play a role in the development, growth or progression of hepatocellular carcinoma (HCC). Genotype and serum titer of HCV also affect the occurrence of HCC in chronic hepatitis C. In this study, we were to evaluate the effects of genotype or serum titer of HCV on the expression of TGF 1. We also intended to examine the correlation between the up-regulation of TGF 1 and the association with HCC in patients with chronic hepatitis C.*

Methods : *We studied 19 patients with chronic hepatitis C and 18 with HCC associated with HCV infection. HCV genotype was determined by line probe reverse hybridization assay and the amount of HCV-RNA was quantitated by branched DNA signal amplification assay. Serum TGF 1 level was measured by enzyme linked immunosorbent assay.*

Results : *HCV genotypes of patients with HCC were similar to those without it. Serum HCV-RNA titer was higher in genotype 1b than in non-1b ($p < 0.05$). Serum TGF 1 levels were higher in HCC than in chronic hepatitis ($p < 0.05$). However, there was no significant difference in the serum TGF 1 level between genotype 1b and non-1b. Also, it was not correlated with the serum HCV-RNA titer or alanine aminotransferase levels.*

Conclusion : *TGF 1 seems to be overexpressed in HCC compared to that of chronic hepatitis C; it was not affected by serum ALT levels, genotype or serum HCV titer. It is suggested that TGF 1 may be associated with the malignant transformation of hepatocyte or the progression of HCV-associated HCC.*

Key Words : *Transforming growth factor beta; Carcinoma, Hepatocellular; Hepatitis C, Chronic*

INTRODUCTION

Transforming growth factor beta-1 (TGF 1) is a cytokine derived from various cells, including leukocytes, Kupffer cells and hepatic lipocytes^{1,2}. It has regulatory roles in cell growth and differentiation^{1,2} and it has been reported to play a key regulatory role in the process of hepatic fibrogenesis by promoting the synthesis of extracellular matrix proteins, collagen and

fibronectin^{4,5}. It has also been suggested that TGF 1 may have an important role in the development, growth or progression of tumors by the fact that it is up-regulated in the cultured cell lines or tissues of several tumors^{6,7}.

Genotype and serum titer of hepatitis C virus (HCV) have been reported to affect the clinical presentations of patients with chronic hepatitis C in terms of the responsiveness to interferon therapy⁸⁻¹⁰, progression of chronic hepatitis and occurrence of hepatocellular carcinoma (HCC)¹¹⁻¹⁴.

In this study, we were to evaluate whether different genotype or serum titer of causes any difference in

*Address reprint requests to : Young-Hwa Chung, M.D.,
Asan Medical Center, 388-1 Pungnap-dong, Songpa-gu,
Seoul 138-736, Korea.*

the expression of serum TGF β 1. We also intended to examine the correlation between the up-regulation of TGF β 1 and the development or progression of HCC in patients with chronic hepatitis C.

PATIENTS AND METHODS

1. Patients

We studied 19 patients with chronic hepatitis and 18 with HCC associated with chronic HCV infection. All patients with chronic hepatitis had elevated serum aminotransferase values for more than 6 months, and also had HCV-RNA in their sera. Patients with serum HBsAg and those with a history of alcohol (over 40 g/day in males, 20 g/day in females over 10 years) or drug abuse were excluded. HCC was diagnosed histologically or based on the findings of hypervascular liver masses on CT scan with serum alpha-fetoprotein levels exceeding 400 ng/mL.

Their clinical records were reviewed for demographic and serum biochemical data (Table 1). The mean age of patients with HCC appeared to be 10 years older than those with chronic hepatitis. Also, serum albumin levels were lower and prothrombin time tended to be more prolonged in HCC than in chronic hepatitis, suggesting that the hepatocellular function of HCC patients may be more deteriorated compared to those with chronic hepatitis.

2. Genotyping and Quantitation of HCV-RNA

Total cellular RNA was extracted from serum by single-step acid guanidinium thiocyanate method with modifications^{15,16)} using RNA STAT-60 (TEL-TEST

Table 1. Clinical characteristics of patients with hepatocellular carcinoma and chronic hepatitis associated with HCV infection

Characteristics	HCC (n=18)	Chronic hepatitis C (n=19)	Total (n=37)	p-value
Age (years)	66±7	53±13	59±12	< 0.01
Sex (MF)	11/7	12/7	23/14	NS
ALT (IU/L)	101±86	130±68	116±133	NS
Albumin (g/dL)	3.6±0.5	4.1±0.6	3.8±0.8	NS
Bilirubin (mg/dL)	1.5±1.4	1.1±0.9	1.3±0.5	NS
Prothrombin time (%)	80±17	91±16	86±17	NS

Data expressed as mean±S.D.

"B", Friendswood, TX). Using products of reverse transcription polymerase chain reaction of 5' untranslated region (5'UTR), HCV genotypes were determined by line probe reverse hybridization assay^{17,18)} (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium).

The amounts of HCV RNA in the sera were quantitated by branched DNA (bdNA) signal amplification assay¹⁹⁾ (Quantiplex, Chiron Diagnostics, Emeryville, CA).

3. Measurement of Serum TGF β 1

Serum TGF β 1 levels were measured by enzyme linked immunosorbent assay (ELISA) using a commercial kit (TGF β 1 ELISA system, Promega, Madison, WI, USA).

4. Statistics

The values of serum HCV-RNA and TGF β 1 were transformed to logarithmic scale before analysis. The results represented as mean±SD were compared by Student's t test. Chi square test or Fisher's exact test was used to evaluate the differences in proportion. Spearman's rank correlation coefficient tested the correlation between the variables. p value < .05 was considered as significant.

RESULTS

1. Genotypes of HCV

Among 37 patients with chronic hepatitis or HCC associated with chronic HCV infection, 26 (70%) were infected with HCV of genotype 1b, 8 (22%) with non-1b and 3 (8%) with both. HCV genotypes of patients with HCV-related HCC were quite similar to those with chronic hepatitis (genotype 1b: 67% vs. 74%; p=NS).

2. Serum Titers of HCV-RNA

In 15 out of 37, the serum titers of HCV RNA were below 0.2 MEq/mL, which is the cut-off value of bdNA assay. The serum titers of HCV RNA tended to be higher in patients with HCC than those with chronic hepatitis (log value, MEq/mL; 1.05±0.98 vs. 1.61±0.92), although it did not reach the statistical significance (p=0.06). Moreover, the proportion of patients with HCV RNA levels > 0.2 MEq/mL was

EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA-1 IN CHRONIC HEPATITIS AND
HEPATOCELLULAR CARCINOMA ASSOCIATED WITH HEPATITIC C VIRUS INFECTION

higher in patients with HCC than in those with chronic hepatitis (78% vs. 42%; $p < 0.05$) (Figure 1). This trend was observed regardless of the genotype of HCV. The amount of serum HCV RNA tended to be higher in genotype 1b compared to genotype non-1b (> 0.2 MEq/mL; 65% vs. 25%; $p < 0.05$) (Figure 2).

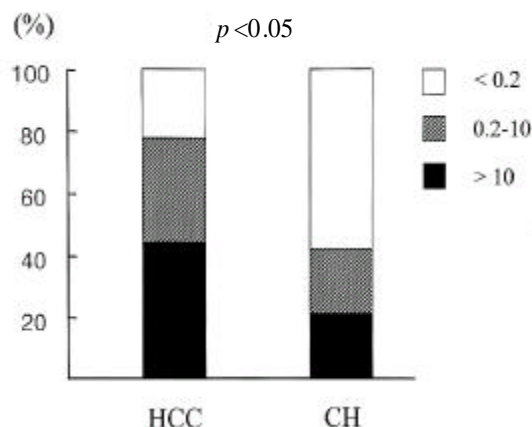


Figure 1. Comparison of quantities of serum HCV RNA in patients with hepatocellular carcinoma to those with chronic hepatitis. The proportion of patients with HCV RNA levels (< 0.2 MEq/mL, 0.2- 10 MEq/mL and > 10 MEq/mL) in HCC and chronic hepatitis was as follows (22%, 34%, 44% vs. 58%, 21%, 21%; $p < 0.05$).

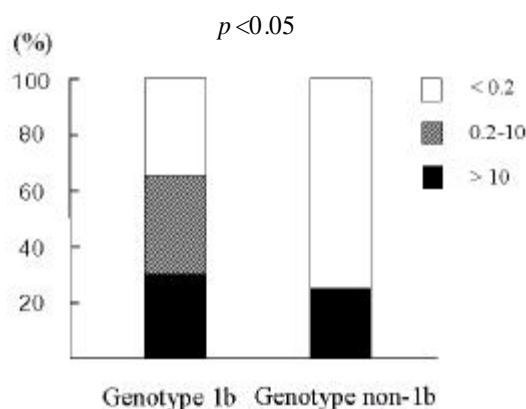


Figure 2. Quantities of serum HCV RNA between the patients with HCV genotype 1b and non-1b. The proportion of HCV RNA levels (< 0.2 MEq/mL, 0.2- 10 MEq/mL and > 10 MEq/mL) between the patients with genotype 1b and non-1b was as follows: 35%, 35%, 30% vs 75%, 0%, 25%; $p < 0.05$.

3. Expression of Serum TGF β 1

Serum TGF β 1 levels were higher in patients with HCC than in those with chronic hepatitis (log value, pg/mL; 2.48 ± 0.25 vs. 2.64 ± 0.22 ; $p < 0.05$) (Figure 3). However, they were not different between genotype 1b and non-1b (log value pg/mL; 2.56 ± 0.26 vs. 2.56 (0.23; $p = NS$) (Figure 4). Serum TGF β 1 levels were not correlated with serum HCV-RNA titers or serum ALT levels ($R = 0.00$ and $R = 0.01$, respectively) (Figure 5).

DISCUSSION

TGF β 1 appeared to be overexpressed in patients with HCC compared to those with chronic hepatitis in this study. It suggests that TGF β 1 may have a role in the development or progression of HCC in patients with chronic hepatitis C. However, serum TGF β 1 level was not different among patients infected with various genotypes of HCV. Also, it was not correlated with serum HCV-RNA titers or ALT levels in patients with chronic HCV infection.

Our data indicated that higher serum titer of HCV-RNA may be more intimately related to genotype 1b as well as the development of HCV-associated HCC. This finding correlates with the previous reports¹¹⁻¹⁴⁾ that high titer of serum HCV-RNA associated

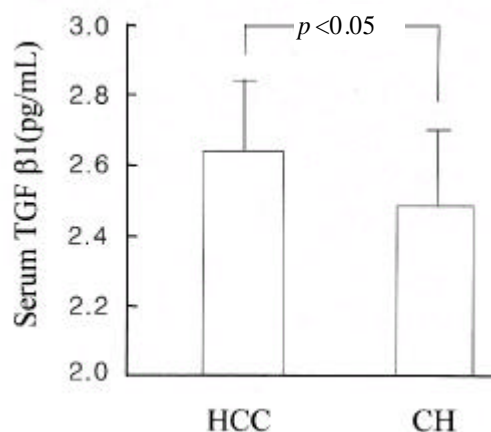


Figure 3. Serum TGF β 1 levels in patients with HCC (n=18) and chronic hepatitis (n=19) associated with chronic HCV infection (log value, pg/mL). Serum TGF β 1 levels were higher in patients with HCC than in those with chronic hepatitis (2.48 ± 0.25 vs. 2.64 ± 0.22 ; $p < 0.05$).

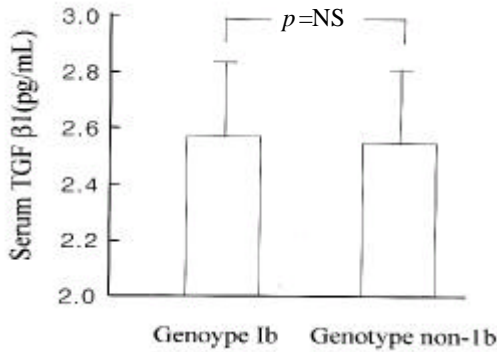


Figure 4. Serum TGF β1 levels in patients with HCC (n=18) and chronic hepatitis (n=19) associated with HCV genotypes (log value, pg/mL). Serum TGF β1 levels in patient with HCV genotype 1b were not different from non-1b (2.56 ± 0.26 vs. 2.56 ± 0.23; p=NS).

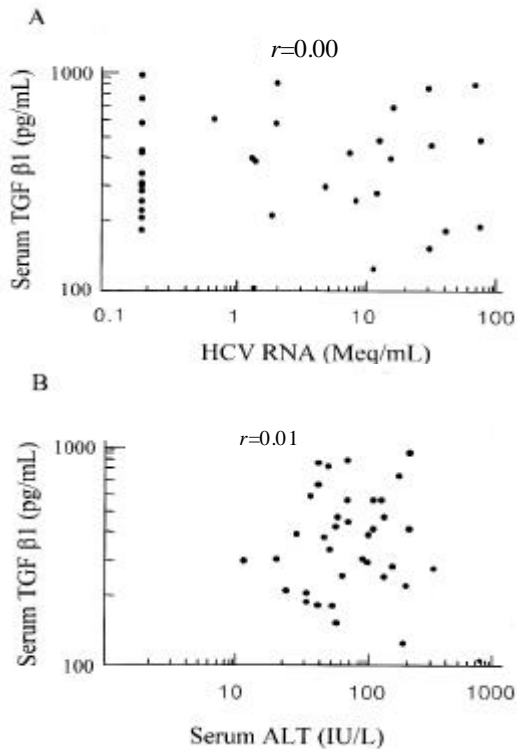


Figure 5. Correlation of serum TGF β1 and HCV RNA levels and ALT levels. Serum TGF β1 levels were not correlated with serum HCV RNA titers (A) or serum ALT levels (B) (r=0.00 and r=0.01, respectively).

frequently with HCC in patients with chronic hepatitis C, and that, especially, genotype 1b had higher serum HCV-RNA level and induced more rapid progression to cirrhosis and HCC. However, in our

data, regardless of its genotype, serum HCV-RNA titer of patients with HCC was higher compared to chronic hepatitis, suggesting that the serum titer of HCV-RNA may be more important than the genotype of HCV in the development of HCC.

Interestingly, there was no correlation between the amounts of TGF β1 and the levels of HCV-RNA in the sera of patients with HCC despite the fact that both of them were higher than in those of chronic hepatitis. This finding indicates that TGF β1 may exert effects on the process of hepatocarcinogenesis different from those of active HCV replication in patients with chronic hepatitis C. A previous report²⁰⁾ suggested that TGF β1 may have much more of a role in the early stage of hepatocarcinogenesis. On the other hand, mutation of p53 gene was more frequently found in the poorly differentiated, multinodular and large-sized HCCs²¹⁾. Recently, there have been some reports^{22,23)} that vascular endothelial growth factor (VEGF) is also associated with the poorly differentiated or advanced stage of HCC. Thus, various growth factors, including TGF β1 and VEGF or genetic alterations such as p53 mutation may have a role in the development or progression of HCC at the different stages of carcinogenesis. Active replication of HCV has been reported to activate various immunocytes in liver tissue followed by the release of various growth factors, which are involved in the transformation of hepatocytes or the progression of tumor cells²⁴⁻²⁷⁾. Clinically, HCV-associated HCC has been known to be different from the HBV-associated tumor in terms of the mean age of patients, the type of tumors and the pathology of the underlying liver²⁸⁾. Thus, it is suggested that the effects of HCV on the alteration of tumor-related gene and the release of various growth factors may be quite different from HBV²⁹⁻³¹⁾. However, the exact role of HCV in the process of development of HCC remains to be clarified.

Our data also showed that serum TGF β1 level was variable even in cases with HCC but we could not evaluate whether it is overexpressed in a certain type of tumor or in a specific clinical presentation of HCC patients because of the small number of subjects. The difference of TGF β1 expression according to the clinical and oncological characteristics needs to be evaluated in the future. Furthermore, it is also necessary to clarify the exact mechanisms of TGF β1 in

EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA-1 IN CHRONIC HEPATITIS AND
HEPATOCELLULAR CARCINOMA ASSOCIATED WITH HEPATITIC C VIRUS INFECTION

hepatocarcinogenesis, including the expression of TGF

1 mRNA in HCC and surrounding liver tissues, the changes of TGF 1 receptor in cell membrane and the response of hepatocytes and interstitial cells to overexpressed TGF 1.

In conclusion, TGF 1 was overexpressed in HCC compared with chronic hepatitis C. However, it was not affected by HCV genotype or serum HCV- RNA titer. It is suggested that TGF 1 may be associated with the malignant transformation of hepatocyte or the progression of HCV-associated HCC.

REFERENCES

1. Sporn MB, Roberts AB, Wakefield LM, Assoian RK. *Transforming growth factor-beta: biological function and chemical structure. Science* 233:532-534, 1986
2. Sporn MB, Roberts AB, Wakefield LM, de Crombrughe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. *J Cell Biol* 105:1039-1045, 1987
3. Ignatz RA, Massague J. *Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem* 261:4337-4345, 1986
4. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UL, Liotta LA, Falanga V, Kehrl JH. *Transforming growth factor-beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci USA* 83:167-171, 1986
5. Olaso E, Friedman L. *Molecular regulation of hepatic fibrogenesis. J Hepatol* 29:836-847, 1998
6. Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH. *Synthesis of RNAs for transforming growth factor ad and the epidermal growth factor receptor by human tumor. Cancer Res* 47:707-712, 1987
7. Zhang X, Wang T, Batist G, Tsao MS. *Transforming growth factor 1 promotes spontaneous transformation of cultured rat liver epithelial cells. Cancer Res* 54: 622-628, 1994
8. Conjeevaram HC, Everhart JE, Hoofnagle JH. *Predictors of a sustained biochemical response to interferon alpha therapy in chronic hepatitis C. Hepatology* 22: 1326-1329, 1995
9. Martinot-Peignoux M, Marcellin P, Pouteau M, Castelnau C, Boyer N, Poliquin M, Degott C, Descombes I, V Le Breton V, Milotova V. *Pretreatment HCV RNA levels and HCV genotype are the main and independent prognostic factors of sustained response to alpha interferon therapy in chronic hepatitis C. Hepatology* 22:1050-1056, 1995
10. Mizokami M, Orio E, Gibo Y, Suzuki K, Ohba K, Ohno T, Lau JY. *Genotype, serum level of hepatitis C virus RNA and liver histology as predictors of response to interferon alpha 2a therapy in Japanese patients with chronic hepatitis C. Liver* 16:23-27, 1996
11. Noursbaum JB, Pol S, Nalpas B, Landais P, Berthelet P, Brechot C. *Hepatitis C virus type 1b infection in France and Italy. Ann Intern Med* 122:161-168, 1995
12. Gretch D, Corey L, Wilson J, de la Rosa C, Wilson R, Carithers R Jr, Busch M, Hart J, Sayers M, Han J. *Assessment of hepatitis C virus RNA levels by quantitative competitive RNA polymerase chain reaction: high-titer viremia correlates with advanced stage of disease. J Infect Dis* 169:1219-1225, 1994
13. Hatzakis A, Katsoulidou A, Kakkamani E, Toubumi G, Koumantaki Y, Tassopoulos NC, Karvountzis G, Gioustozi A, Hadziyannis S, Trichopoulos D. *Hepatitis C virus 1b is the dominant genotype in HCV-related carcinogenesis: a case-control study. Int J Cancer* 68: 51-53, 1996
14. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, Asti M, Rossi S, Larghi A, Cerino A, Podda M, Mondelli MU. *Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. Hepatology* 25:754-758, 1997
15. Chomczynski P, Sacchi N. *Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem* 162:156-159, 1987
16. Puissant C, Houdobine L. *An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Biotechniques* 8:148-149, 1991
17. Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS. *Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol* 74: 2391-2399, 1993
18. Stuyver L, Rossau R, Wyseur A, Duhamel M, Vanderborgh B, Van Heuverswyn H, Maetens G. *Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. J Gen Virol* 74:1093-1102, 1993
19. Urdea MS, Horn T, Fultz TJ, Anderson M, Running JA, Hamren S, Ahle D, Chang CA. *Branched DNA amplification multimers for the sensitive, direct detection of human hepatitis virus. Nucleic Acids Symp series* 24:197-200, 1991
20. Song BC, Chung YH, Yang SH, Kim JA, Hong IR,

- Lee JK, Jung SA, Lee YJ, Lee SK, Lee YS, Suh DJ. Expression of transforming growth factor- α mRNA in hepatocellular carcinoma and surrounding liver. *Korean J Gastroenterol* 34:774-783, 1999
21. Youn KH, Chung YH, Yang SH, Song BC, Hong IR, Kim JA, Lee YS, Suh DJ, ES Yu, Le YJ, Lee SK. Correlation of p53 mutation and microvascular invasions of hepatocellular carcinoma: a possible factor of poor prognosis following surgical resection. *Korean J Hepatol* 5:124-135, 1999
22. Torimura T, Sata M, Ueno T, Kin M, Tsuji R, Suzaku K, Hashimoto O, Sugawara H, Tanikawa K. Increased expression of vascular endothelial growth factor is associated with tumor progression in hepatocellular carcinoma. *Human Pathol* 29:986-991, 1998
23. Jinno K, Tanimizu M, Hyodo I, Nishikawa Y, Hosokawa Y, Doi T, Endo H, Yamashita T, Okada Y. Circulating vascular endothelial growth factor (VEGF) is a possible tumor marker for metastasis in human hepatocellular carcinoma. *J Gastroenterol* 33:376-382, 1998
24. Tarao K, Ohkawa S, Shimizu A, Harada M, Nakamura Y, Ito Y, Tamai S, Hoshino H, Inoue T, Kanisawa M. Significance of hepatocellular proliferation in the development of hepatocellular carcinoma from anti-hepatitis C virus-positive cirrhotic patients. *Cancer* 73:1149-1154, 1994
25. Nardone G, Romano M, Calabro A, Pedone PV, de Sio I, Persico M, Budillon G, Bruni CB, Riccio A, Zarilli R. Activation of fetal promoters of insulin-like growth factor II gene in hepatitis C virus-related chronic hepatitis, cirrhosis and hepatocellular carcinoma. *Hepatology* 23:1304-1312, 1996
26. Tanaka S, Takenaka K, Matsumata T, Mori R, Sugimachi K. Hepatitis C virus replication is associated with expression of transforming growth factor- α and insulin-like growth factor -II in cirrhotic liver. *Dig Dis Sci* 41:208-215, 1996
27. Tsai JF, Jeng JE, Chuang LY, Chang WY, Tsai JH. Urinary transforming growth factor beta I levels in hepatitis C virus-related chronic liver disease: correlation between high levels and severity of disease. *Hepatology* 25:1441-1446, 1997
28. Okuda K. Hepatitis C virus and hepatocellular carcinoma. In: Okuda K, Tabor E, eds. *Liver Cancer*. 1st ed. p. 39-50, New York, Churchill Livingstone, 1997
29. Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblast to tumorigenic phenotype. *J Virol* 70:4438-4443, 1995
30. Kim SO, Park JK, Lee YI. Increased expression of the insulin-like growth factor I (IGF-I) receptor gene in hepatocellular carcinoma cell lines: implication of IGF-I receptor gene activation by hepatitis B virus X gene product. *Cancer Res* 56:3831-3836, 1996
31. Jakubczak JL, Chisari FV, Merlino G. Synergy between transforming growth factor and hepatitis B virus surface antigen in hepatocellular proliferation and carcinogenesis. *Cancer Res* 57:3606-3611, 1997