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Enhanced nuclear factor-kappa B-associated *Wnt-1* expression in hepatitis B- and C-related hepatocarcinogenesis: identification by functional proteomics

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Summary

Chronic infections with hepatitis B and C viruses (HBV and HCV) are etiologically linked to hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Both viruses may induce activation of nuclear factor-kappa B (NF-κB) in hepatocytes that plays a crucial role in the regulation of cell growth and apoptosis. Functional proteomics analysis of proteins associated with NF-κB signaling complexes in both viruses-related HCC tumor and non-tumor tissues may disclose possible common mechanisms in hepatocarcinogenesis. By functional proteomics, we analyzed proteins associated with NF-κB-signaling complexes in four-paired human HCC tumor and non-tumor tissues from HBV- and HCV-infected patients, respectively, and in one-paired tissue with dual viral infection. The quantity of NF-κB-associated proteins was semi-quantitatively measured by protein spot intensity on the gels of two-dimensional polyacrylamide gel electrophoresis. The results showed that overexpression of NF-κB-associated *Wnt-1* protein in tumor part was detected in the majority of HBV- and HCV-infected HCC samples. These data suggest that enhanced expression of NF-κB-associated *Wnt-1* protein might be a mechanism of hepatocarcinogenesis common to HBV- and HCV-infected patients. NF-κB signaling pathway and *Wnt-1* protein could be potential targets for designing highly effective therapeutic agents in treating HCC and for chemoprevention of hepatocarcinogenesis.

Abbreviations: m/z – mass-to-charge ratio; 2-DE – two-dimensional polyacrylamide gel electrophoresis; EMSA – electrophoretic mobility shift assay; HBV – hepatitis B virus; HCC – hepatocellular carcinoma; HCV – hepatitis C virus; HSP – heat shock protein; MALDI-Q-TOF – matrix-assisted laser desorption/ionization-quadrupole-time-of-flight; MS – mass spectrometry; NF-κB – nuclear factor-kappa B

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Introduction

The hepatitis B and C viruses (HBV and HCV) infect more than 350 and 170 million people worldwide, respectively [1]. Both share common clinical manifestations in chronically infected subjects including similar histopathological changes in the liver, common clinical evolution from chronic hepatitis, liver cirrhosis and ultimately to hepatocellular carcinoma (HCC) [2–4]. Accumulating evidence indicates that both HBV [5–9] and HCV [10–14] may induce activation of nuclear factor-kappa B (NF-κB). Constitutive and/or inducible activation of NF-κB has been established in HBV-positive cell line Hep3B [15] and HCV-transfected HepG2 cells [13], as well as in HBV- and HCV-infected liver tissues [13, 16]. The activated NF-κB could be demonstrated by immunohistochemical staining, electrophoretic mobility shift assay (EMSA) and supershift assay. The importance of NF-κB in immunity is undisputed [17, 18]. Recent evidence indicates that NF-κB and its activation pathways are also important for tumor development [19–22]. These features prompt us to propose that there exist possible mechanisms of NF-κB-related hepatocarcinogenesis common to both viruses. To test this hypothesis, we analyzed NF-κB activation in four-paired human HCC tumor and non-tumor tissues from HBV- and HCV-infected patients, respectively, and in one paired sample with dual viral hepatitis B and C. Proteins associated with NF-κB signaling complexes were studied by functional proteomics approach [23, 24].

Materials and methods

The scheme of study procedures is outlined in Figure 1. Proteins were extracted from nine pairs

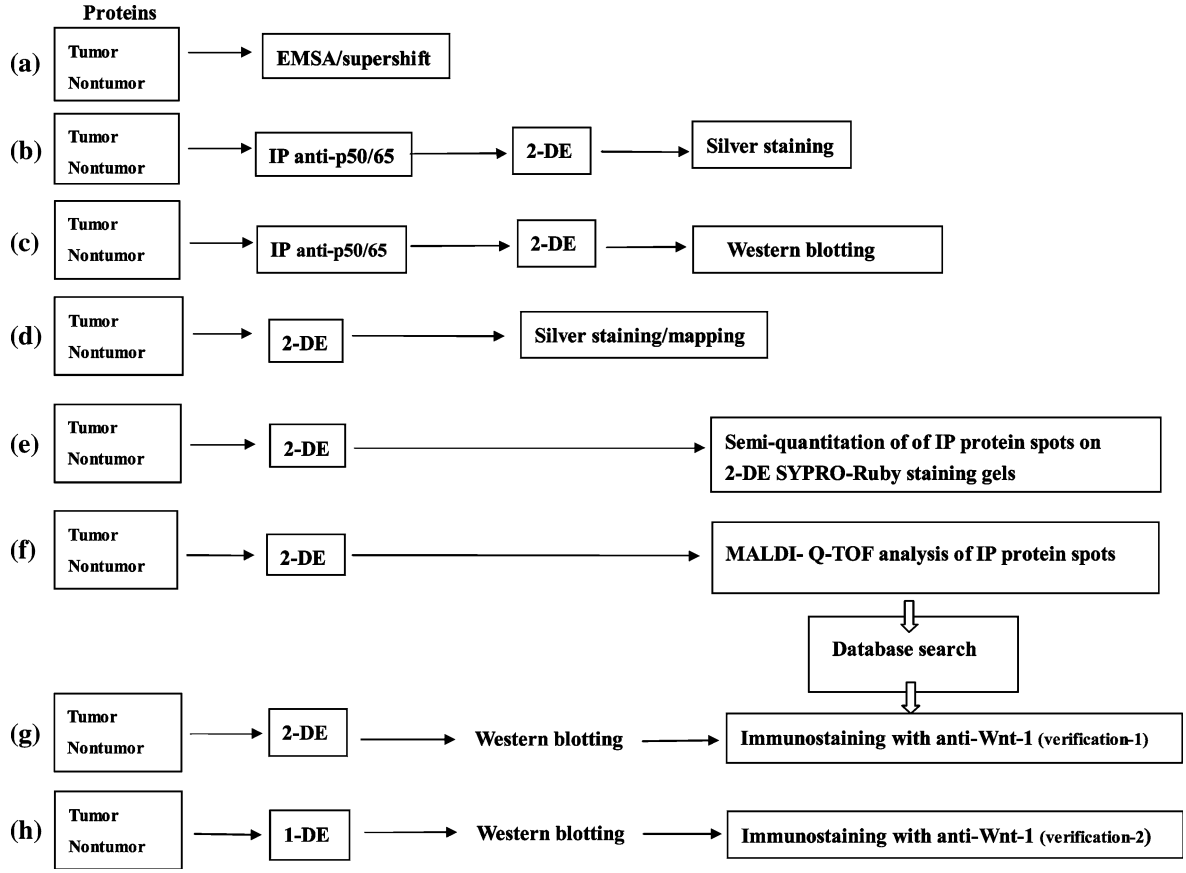


Figure 1. The scheme of study procedures. Proteins were extracted from liver tissues that were separated into tumor and non-tumor parts for each pair of samples. EMSA, electrophoretic mobility shift assay; IP, immunoprecipitation; 2-DE, two-dimensional polyacrylamide gel electrophoresis; MALDI-Q-TOF, matrix-assisted laser desorption/ionization-quadrupole-time-of-flight.

of HCC liver tissues, which were separated into tumor and non-tumor portions for each pair of sample. Two parts of study were conducted in parallel: the first part was run individually for each paired-sample divided into tumor and non-tumor portions, the second part was carried out on pooled total tumor proteins and on pooled total non-tumor proteins of the 9 paired-samples.

Sample preparation

Fresh tissue specimens of nine HCC patients who received surgical treatment of liver tumors in Chi-Mei Medical Center (Tainan, Taiwan) were separated into tumor and non-tumor portions immediately after operation. The clinical features of these nine patients are summarized in Table 1. Additional eight-paired HCC samples from Tumor and Serum Bank of Chi-Mei Medical Center were studied in parallel for the verification. Informed consent was obtained from each of all study subjects for the donation of their liver tissues and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Individual liver tissue specimen from each patient was homogenized in lysis buffer using a Potter-type homogenization instrument. A 0.2 g of liver in 5 ml homogenization buffer gave 5–10 mg/ml final protein concentrations. Unbroken cells and connective tissue were removed from the homogenate by centrifugation at 250× *g* for 10 min at 4 °C. The samples were kept on ice at all times and the supernatants were stored at −70 °C until use. Protein quantity was determined using a Plus-One 2D Quant Kit (Amersham Biosciences Corp., Piscataway, NJ, USA).

EMSA and supershift assay

Nuclear and cytoplasmic extracts were prepared according to standard protocols [25]. EMSA and supershift assay of NF-κB on tumor and non-tumor parts were performed by using procedures described previously [13, 16].

Immunoprecipitation (IP) of NF-κB-associated protein complexes

HCC tumor and non-tumor proteins 500 μg each in rehydration buffer (7 M Urea, 2 M Thiourea, 4% CHAPS, 0.5% IPG buffer and a few Bromophenol

Table 1. Patient characteristics.

Patient No.	Age (year)	Gender	Tumor		Clinical stage (Child-Pugh)	Virus	Background	Operation	Increase of *Wnt-1* protein expression (Fold)
			Stage	Histopathology[a]					
1.	50	F	I	poor-HCC	A	HCV	CH		3.5[b]
2.	60	M	I	well-HCC	A	HCV	AC		0.9
3.	53	M	I	poor-HCC	A	HCV	AC	Egmentectomy	3.0
4.	62	F	II	mod-HCC	A	HCV	LC	Egmentectomy	2.6
5.	75	M	II	well-HCC	A	HBV	CH	Egmentectomy	1.4[b]
6.	49	M	II	poor-HCC	A	HBV	AC	Egmentectomy	2.7
7.	51	F	I	well-HCC	A	HBV	LC	Egmentectomy	2.1
8.	72	M	II	mod-HCC	A	HBV	LC	Egmentectomy	2.2
9.	55	M	II	poor-HCC	B	HBV + HCV	LC	Rt lobectomy	11.4[c]

[a]well-HCC, well-differentiated hepatocellular carcinoma; mod-HCC, moderately differentiated HCC; poor-HCC, poorly differentiated HCC; HBV, hepatitis B virus; HCV, hepatitis C virus; LC, liver cirrhosis; AC, active cirrhosis; CH, chronic hepatitis.
[b]Figure 6 and supplementary data A.
[c]Figure 5.

blue) were separately mixed with anti-human p50 and p65 of NF-κB (Biogenesis, Poole, UK) for 1.5 h at 4 °C. The immunocomplexes were collected by Protein A Sepharose^{TM}CL-4B beads (Amersham Biosciences) according to manufacturers' instructions.

Two-dimensional polyacrylamide gel electrophoresis (2-DE)

Two-dimensional polyacrylamide gel electrophoresis (2-DE) was carried out according to standard protocols [26], and the detailed procedures as described previously [27].

Gel staining and image analysis

Silver staining. A modified silver staining method compatible with mass spectrometric analysis provided protein spot visualization was used for the study [27]. Briefly, gels were fixed in 50% methanol, 10% acetic acid in water for 30 min followed by incubation in 5% methanol for 15 min. The gels were washed three times in Milli-Q water for 5 min each and then sensitized with freshly prepared 0.02% sodium thiosulphate for exactly 2 min. The sensitized gels were then washed three times with Milli-Q water for 30 s each. The gels were treated with 0.2% silver nitrate for 25 min and rinsed three times in Milli-Q water for 1 min each. The gels were developed with 3% sodium carbonate, 0.018% formaldehyde and 0.02% sodium thiosulphate. The desired intensity of staining was achieved after 3–4 min in the developer and stopped with 1.4% sodium EDTA for 10 min. Gels were then rinsed twice in Milli-Q water for 2 min each.

SYPRO-Ruby staining. The gels were fixed in 10% methanol and 7% acetic acid for 30 min followed by washing three times each with water for 5 min. To obtain the maximum sensitivity, the gels were incubated with SYPRO-Ruby solution (Molecular Probes, Eugene, OR, USA) for at least 3 h. To reduce background fluorescence and increase sensitivity, the stained gels were washed in 10% methanol and 7% acetic acid for 30 min.

Image recording and analysis. The gels were rinsed twice with water for 5 min each, before being scanned on the Typhoon 9200 ImageMaster (Amersham Biosciences). The image analysis and 2-DE gel proteome database management were performed using the ImageMaster 2D Platinum Software, version 5.0 (Amersham Biosciences). The theoretical Mr and pI values of the 2-DE markers were used to calibrate the Mr and pI of the protein spots on the 2-DE gels. Intensity levels were normalized between gels as a proportion of total protein intensity detected for the entire gel and the protein quantity of each spot was calculated by integrating density over the spot area [27, 28].

Mass spectrometric analysis

Tryptic peptides from 2-DE protein spots were subjected to peptide mass fingerprinting (PMF) using a matrix-assisted laser desorption/ionization quadrupole-time of flight (MALDI-Q-TOF) mass spectrometer (M@LDI^{TM}; Micromass, Manchester, UK) operated in reflectron positive ion mode as described [27–29]. Briefly, samples were spotted onto the 96-well format MALDI target plate using a saturated matrix solution of α-cyano-4-hydroxycinnamic acid (CHCA) in 60% ACN/1% TFA. The instrument was externally calibrated with standard peptide mixtures and further adjusted with the lock mass feature using adrenocorticotropic hormone (ACTH) as the near-point calibrant. Mass spectra were acquired for the mass range of 900–3000 Da and automatically processed by the ProteinLynx^{TM} software for PMF searches against the SWISS-PROT database employing the MASCOT program [30]. The search parameters allowed for one missed cleavage, oxidation of methionine, N-terminal acetylation, and carboxyamido-methylation of cysteine. Positive identification of proteins required at least five matching peptide masses with 50 ppm or better mass accuracy.

One-DE and two-DE Western blot analysis of Wnt-1 protein

Analytical 1-DE and 2-DE gels were electrotransferred onto PVDF membranes (Hybond P, Amersham Biosciences) for Western blot analysis of *Wnt-1* protein by the standard procedures using biotin-conjugated rabbit anti-human Wnt-1 antibody (ZYMED Lab. Inc., CA). Reaction with the primary antibody was visualized using an enhanced chemiluminescence detection system

(ECLplus, Amersham Biosciences) and exposed to autoradiography films for 3–15 min. The quantity of *Wnt-1* protein expression was semi-quantitatively estimated on the films using ImageMaster TotalLab, Version 2.01 (Amersham Pharmacia Biotech, NJ).

Results

Constitutive activation of NF-κB in HBV- and HCV-related HCC tumor and non-tumor tissues. Activation of NF-κB was analyzed by EMSA and supershift assay (Figure 2). Supershift studies for tumor portions of patients 1–7 showed that the activated NF-κB bands in HBV- and HCV-infected livers undergo a supershift with anti-P50 (Figure 2a). Supershift with anti-P65 revealed similar results (data not shown). These data should be carefully interpreted that numerous factors or proteins are related to NF-κB activation [19–21, 31]. Of the nine initial study patients, the enhancement of activated NF-κB bands is variable between tumor and non-tumor portions (Figure 2a and b) and no NF-κB activation in one normal liver without infection with hepatitis viruses (Figure 2c) Thus NF-κB activation in tumor portions is not necessary more prominent than that of non-tumor portions from the same patient. In summary, these results indicate constitutive activation of NF-κB in tumor and non-tumor parts of HBV- and HCV-related HCC samples.

Two-DE of NF-κB-associated protein complexes. Proteins from tumor portions of the nine HCC patients were pooled together, then subjected to anti-P50 IP or anti-p65 IP, followed by 2-DE analysis. Proteome profiles NF-κB-associated protein complexes by anti-P50 IP are shown in Figure 3a, and by anti-p65 IP in Figure 3b. An equivalent 20 spots positively stained on 2-DE gels of anti-p50 IP and/or anti-p65 IP were arbitrarily selected and mapped together with spots revealed on silver staining of 2-DE gels of the pooled total tumor proteins without IP are shown in Figure 4. After SYPRO-Ruby staining, these 20 spots were forwarded to the treatment of in-gel trypsin digestion and subsequently for mass spectrometric analysis.

Comparison of volume quantity of spot MI205434. We measured individually these 20 spots semi-quantitatively and found interesting differences between tumor and non-tumor tissues, particularly the spot MI205434 with quantities significantly greater in tumor than in non-tumor portions in 7 of 9 paired-samples. The comparison of volume quantity of the spot MI205434 between tumor and non-tumor parts of patient 9 is shown in Figure 5 (for patients 1 and 5 using the same

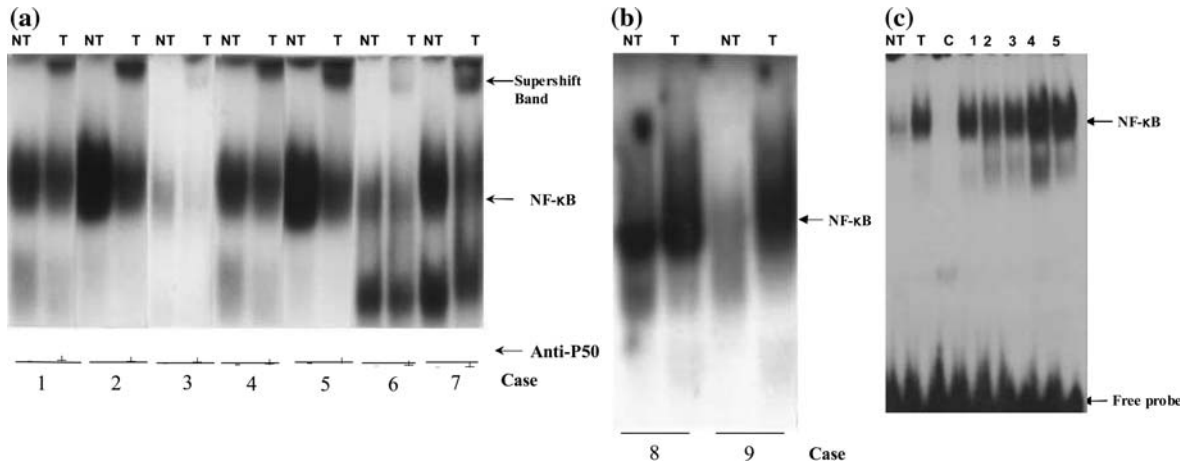


Figure 2. EMSA and supershift assay. (a) Displays results of EMSA and supershift assay on tumor portions probed with anti-p50 of NF-κB analyzed for patients 1–7. (b) Shows EMSA data of patients 8 and 9; and (c) are results of another experiment using one-paired HCC tumor (T) and non-tumor (NT) tissues, normal liver control without HBV or HCV infection from liver biopsy (C) during cholecystectomy of gallbladder stones, and tumor tissues from five (1–5) of the additional eight-paired HCC samples for the verification. The specificity of the shifted bands of NF-κB was ascertained by competition studies [13] with the mutant probe and a 50-fold excess of wild-type cold probe (data not shown).

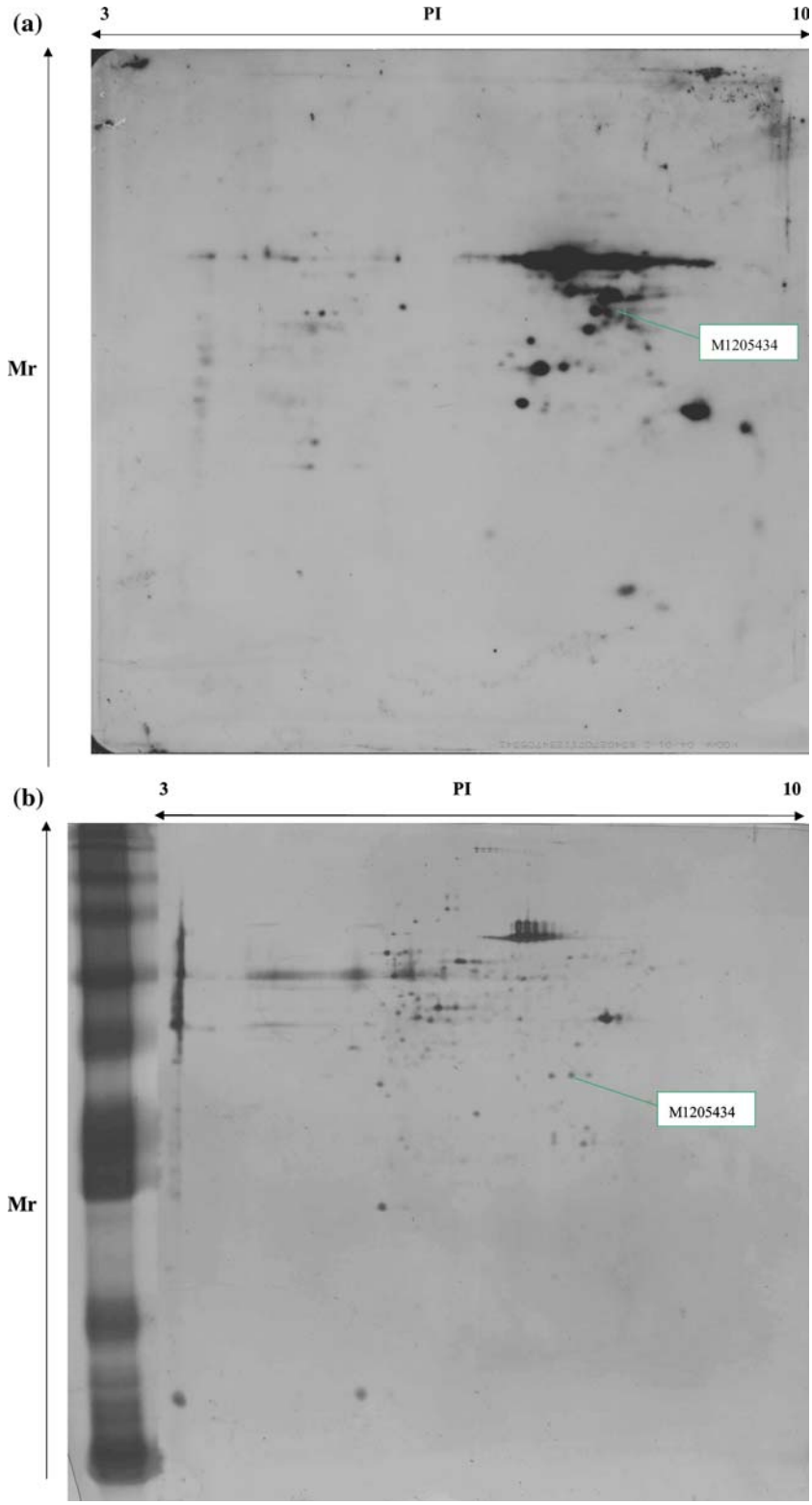


Figure 3. Silver staining of 2-DE gels of pooled HCC tumor proteins after IP. (a) HCC tumor proteins 500 μg from each sample of the 9 patients were processed with IP using anti-P50 of NF-κB, then pooled together for 2-DE, and subsequently for silver staining. (b) IP using anti-P65. The position of spot M1205434 was marked on both figures.

comparison method, see Supplementary data A). The comparison of the spot MI205434 between tumor and non-tumor parts of these 9 study patients is summarized in Table 1.

Mass spectrometric analysis and immunoblot analysis. Mass spectrometric analysis of spot MI205434 and other 19 IP spots (data not shown, see Supplementary data B). Several protein candidates by database search might be the spot MI205434 (Supplementary data C). However, the integration of database search results of peptide mass fingerprinting of the spot MI205434 (Table 2), 2-DE Western blot analysis with anti-human *Wnt-1* (Figure 6), and 1-DE Western blot analysis of additional eight-paired samples from patients 10–17 (Figure 7), suggests that the candidate protein for the spot MI205434 is most likely *Wnt-1* protein. The data shown in Figure 7, revealed higher levels of tumor *Wnt-1* expression in terms of more than two-fold increase, in 6 of 8 patients studied, comparable to the volume of tumor spot MI205434 expression increased in 7 of 9 initial study patients estimated directly from 2-DE gels (Table 1). The highest enhancement (11.4 times) of spot MI205434 in tumor portion of the initial 9 study patients was detected in the patient 9 with dual hepatitis B and C (Figure 5). In summary, our study showed more prominent expression of NF-κB associated *Wnt-1* protein in HCC tumor than in non-tumor portions from both HBV- and HCV-related HCC patients.

Discussion

This study analyzed proteins associated with NF-κB signaling complexes in HCC tumor and non-tumor tissues by functional proteomics approach.

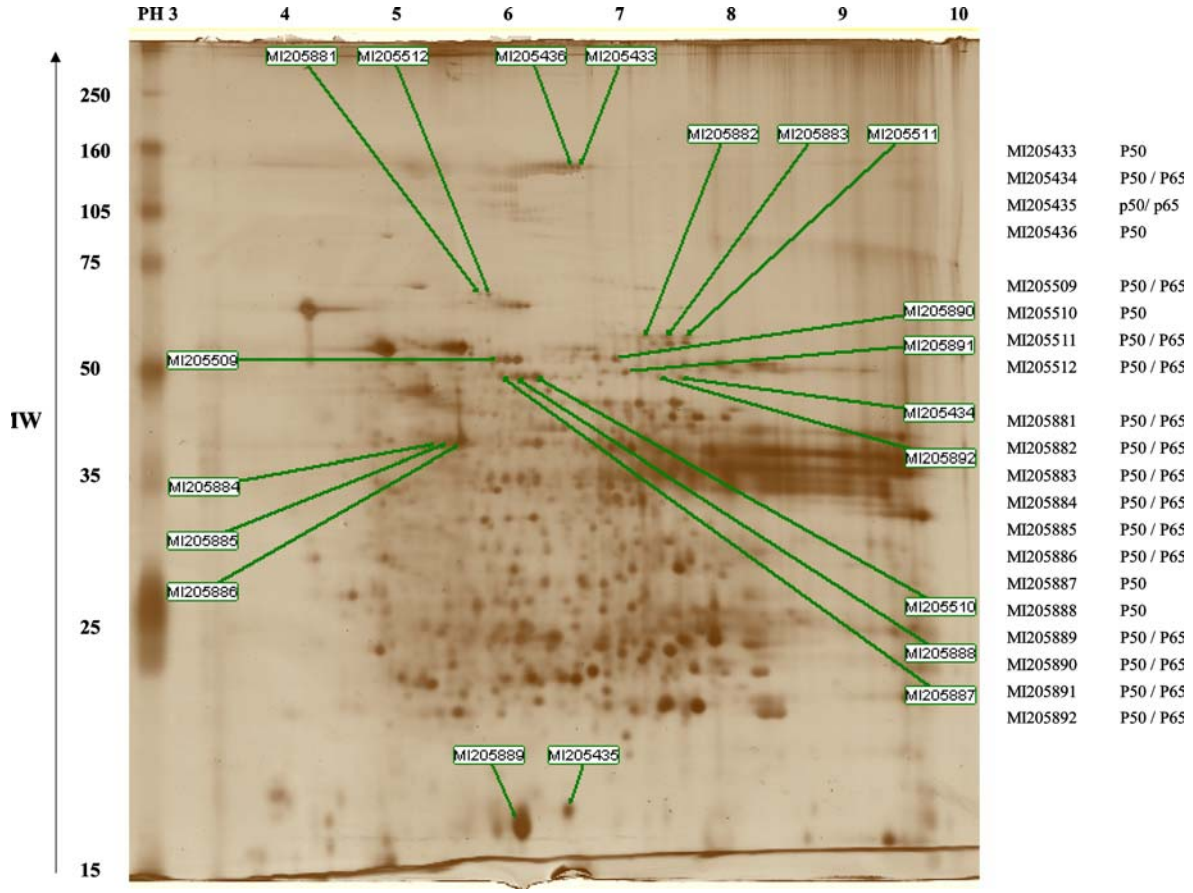
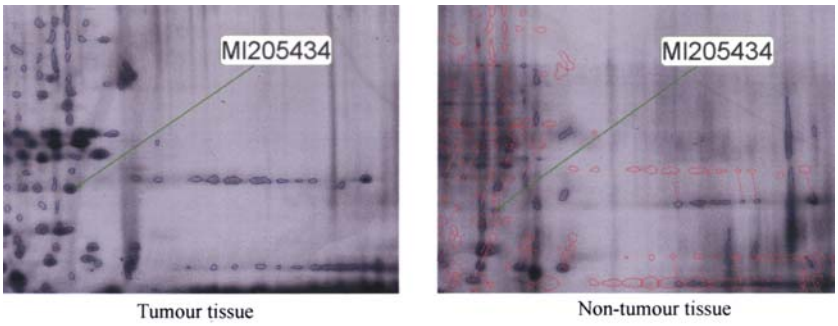


Figure 4. Mapping of IP and 2-DE silver staining of pooled HCC tumor proteins. An equivalent 20 spots positive for anti-p50 and/or anti-p65 were arbitrarily selected and mapped together using ImageMaster. The numbers of 20 spots shown in the right side of the figure were arbitrarily selected for MALDI-Q-TOF analysis.



Gel	Source Gel	Spot	Volume : IOD (CV)	Fold	Area : Pixels (CV)	Fold
Tumour		500	236.057	11.408	376	4.13
Non-tumour		346	20.692		91	

Figure 5. Volume comparison of spot M1205434 between tumor and non-tumor tissue of patient 9. The 20 spots shown in Figure 4 were mapped for each patient. Then volume comparison of spot M1205434 was made by ImageMaster between tumor and non-tumor tissue for the 9 study patients. The result of patient 9 is shown. Numbers on the spot column were automatically nominated.

NF-κB-associated *Wnt-1* protein expression was incidentally identified to increase more in tumor than in non-tumor portions from both HBV- and HCV- related HCC samples. These data suggest that overexpression of NF-κB associated *Wnt-1* protein might be an important mechanism of hepatocarcinogenesis common to HBV and HCV infection. Several studies have reported proteomic analysis of HCC, either from tumor tissues [29, 32, 33], or from patients' sera [34]. All these studies attempted to identify specific factors correlated with hepatocarcinogenesis or novel tumor markers for early diagnosis of HCC, but these goals seem far from reach [35, 36]. Bournat et al. [37] firstly reported the link of proto-oncogenic protein *Wnt-1* with NF-κB activation. Their study showed that the *Wnt-1* mediated survival of PC12 cells, a rat pheochromocytoma cell line of neural crest lineage, is dependent on NF-κB activation, and that stable expression of *Wnt-1* increases NF-κB activity. The association of Wnt signaling with NF-κB signaling pathway had been reported earlier in studying the ubiquitin-dependent proteolysis by the proteasome [38]. The key mediator of that pathway is β-catenin [39]. Moreover, a link of HBV with Wnt signaling has been reported in hepatoma cells that X-protein of HBV may enhance stabilization of β-catenin, and is essential for the activation Wnt/β-catenin signaling [40]. A comprehensive analysis of gene profiles of HBV-related HCC has revealed that gene expression of Wnt/β-catenin pathway is also increased in tumor portions [41]. There is also reported that the hepatocyte growth stimulated by HCV core protein is correlated with upregulation of *Wnt-1* expression [42]. This provides a strong evidence of the association of HCV infection with Wnt/β-catenin signaling. Additionally, indirect evidence from proteomics approach has also identified the heat-shock protein 27 (HSP27), interacting with non-structural protein 5A (NS5A) of HCV [43]. Likewise, HSP70 showed a tendency toward overexpression in HCV-related HCC tumor tissues [44]. The induced heat shock proteins include some that help stabilize and repair partly denatured cell proteins that are closely related to

Table 2. Database search results of peptide mass fingerprint for spot MI205434.*

1	MGLWALLPGW	VSATLLLALA	ALPAALAANS	SGRWWGIVNV	ASSTNLLTDS
51	KSLQLVLEPS	LQLLSRKQRR	LIR**QNPGILH**	**SVSGGLQSAV**	**RECK**WQPRNR
101	**RWNCPTAPGP**	**HLFGK**IVNRG	CRETAPIFAI	TSAGVTHSVA	RSCSEGSIES
151	CTCDYRR**RGP**	**GGPDWHWGGC**	**SDNIDFGRLF**	GREFVDSGEK	GRDLR**FLMNL**
201	**HNNYEAGRTTV**	**FSEMR**QECKC	HGMSGSCTVR	TCWMRLPTLR	AVGDVLRDRF
251	DGASRVLYGN	RGSNRASRAE	LLRLEPEDPA	HKPPSPHDLV	YFEK**SPNFCT**
301	**YSGRLGTAGT**	**AGR**ACNSSSP	ALDGCELLCC	GRGHRTRTQR	VTERCNCTFH
351	WCCHVSCRNC	**THTRVLHECL**			
Start–End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
74–94	2180.05	2179.04	2179.12	−0.08	1	QNPGILHSVSGGLQSAVRECK
102–115	1581.73	1580.72	1580.76	−0.03	0	WNCPTAPGPHLFGK Carbamidomethyl (C)
159–178	2129.99	2128.98	2128.88	0.10	0	GPGGPDWHWGGCSDNIDFGR
196–215	2399.17	2398.16	2398.12	0.05	1	FLMNLHNNEAGRTTVFSEMR 2 Oxidation (M)
295–313	1915.92	1914.91	1914.90	0.01	1	SPNFCTYSGRLGTAGTAGR
359–370	1425.62	1424.61	1424.67	−0.06	1	NCTHTRVLHECL

Score: 45; Expect: 1.4; Wnt-1 proto-oncogene protein precursor; Sequence Coverage: 29%.
*Match to: NCBI/SWISS PROT No. P04628.

ubiquitin-dependent proteolysis pathway [45] and are also linked to NF-κB signaling and cell survival or apoptosis [46]. These data, at least indirectly, suggest a contribution by HCV infection to virus-induced endoplasmic reticulum stress in hepatocytes that is implicated to play a role in hepatocarcinogenesis [47]. The details require further investigation.

Wnt genes encode a family of 38–45 kDa, secreted cysteine-rich proteins lacking transmembrane domains that are modified by N-linked glycosylation [48]. The secreted Wnts associate with extracellular matrix proteins on or near the cell surface, and thus can exert autocrine or paracrine effects. The first member of the 19 known human Wnt genes, Wnt-1, was first discovered because of its oncogenic properties [49]. The subsequent discovery of wingless, the fly homolog of Wnt-1, paved the way for assembling a signaling pathway found to contain cancer-causing genes [48, 50–55]. There have been numerous reports on the overexpression, and sometimes underexpression of Wnt genes in human cancers and on dysregulated *Wnt* signaling in hematological malignancies [51–54]. More compelling evidence, such as amplification, rearrangement, or mutation of genes encoding Wnt ligands or receptors has been increasing, e.g., Wnt mutations were reported to occur in 85% of colorectal cancer, 74% of desmoid tumor, and 67% of hepatoblastoma [51–53]. In the present study, we further demonstrated overexpression of *Wnt-1* protein in both HBV- and HCV-related HCC tumor tissues. Most importantly, it is NF-κB-associated. This finding is consistent with the report by Pikarsky et al. [22] that NF-κB may function as a tumor promoter in inflammation-associated cancer.

Evidence shows that activation of NF-κB inhibits apoptosis and inhibition of NF-κB enhances anti-tumor therapy through increased apoptosis [56–58]. Moreover, it has also been reported that activation of *IEX-1L* gene, an apoptosis inhibitor, and the induction of inhibitor of apoptosis proteins (c-IAP1 and c-IAP2) may be involved in NF-κB-mediated cell survival [59, 60]. Whether NF-κB-associated *Wnt-1* protein expression in HBV- and HCV-infected HCC tumor and non-tumor tissues is related to these anti-apoptosis factors remains to be elucidated.

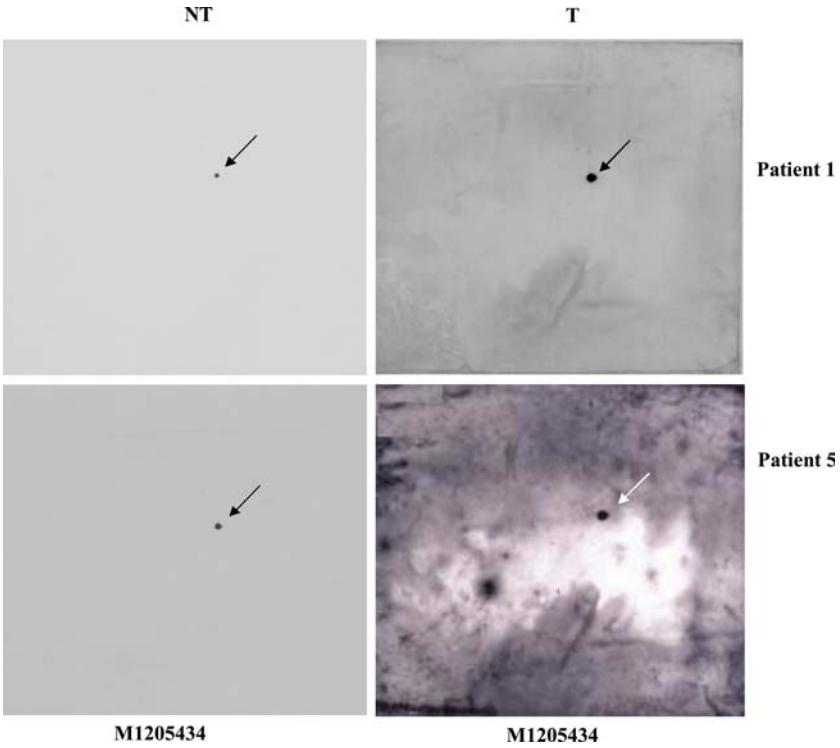


Figure 6. Two-DE Western blotting analysis. The *Wnt-1* protein of spot M1205434 by database search was confirmed by 2-DE Western blotting probed with anti-human Wnt-1 antibodies. A comparison of tumor and non-tumor *Wnt-1* protein expression for patients 1 and 5 is shown.

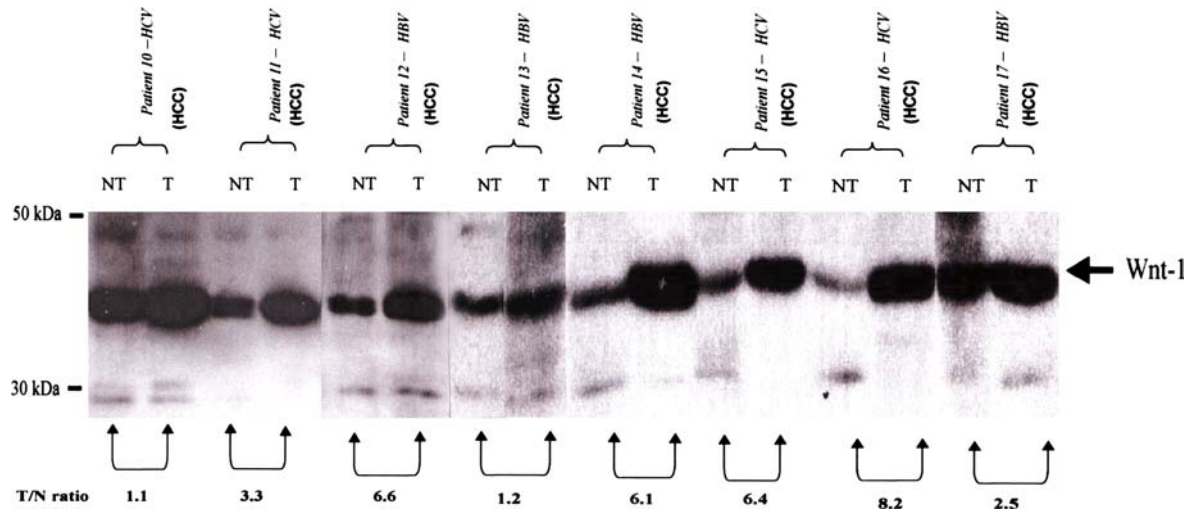


Figure 7. Western blot analysis of additional eight pairs of HCC samples from patients 10–17. Densitometry analysis of *Wnt-1* protein expression on the film of Western blot was made by ImageMaster. Relative intensity of *Wnt-1* protein expressed by tumor tissue (T) to non-tumor tissue (NT) is shown below each pair of sample.

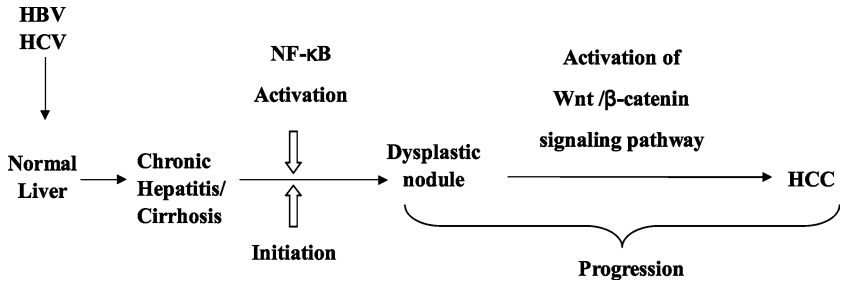


Figure 8. The proposal of this study in terms of the enhanced expression of NF-κB-associated *Wnt-1* protein in hepatocarcinogenesis. The possible scientific basis of this proposal is described in the text.

An overwhelming evidence indicates that the evolutionarily conserved Wnt-signaling pathway has pivotal roles during the development of many organs [48, 53, 54], and dysregulated Wnt-signaling is a key factor in the initiation and of various tumors and the development of diseases [50, 51, 53, 55, 61]. Our data showed that overexpression of NF-κB associated *Wnt-1* is more prominent in HCC tumor parts than in non-tumor parts from both HBV- and HCV-infected patients. This implies that NF-κB associated *Wnt-1* is important to HCC formation. It may serve as a common denominator of HBV- and HCV-related hepatocarcinogenesis. The scenario of this proposal in terms of the enhanced expression of NF-κB-associated *Wnt-1* protein might be illustrated as Figure 8. It is based on the facts that the Wnt proteins (Wnts) are ligands for the Frizzled (*Fz*) receptors, which resemble typical G protein coupled receptors [48, 51]. Consequently, by the mechanism of receptor-mediated endocytosis [51], Wnts signaling can involve intracellularly the regulation of a multiprotein complex including NF-κB. It is conceivable that NF-κB activated by HBV and HCV infections may further interact with Wnts and other regulatory factors to control cell growth.

In view of common manifestations of HBV and HCV infections, our finding is clinically relevant. Although multimodality treatment protocols have been applied to treat HCC patients, up to now the prognosis of this cancer is still very poor [2, 62, 63]. The high mortality associated with HCC is because it is often unresponsive to treatment. An effective treatment regimen is thus pending. It is suggested in children with medulloblastoma [64] and in patients with head and neck squamous cell carcinomas [65] that the Wnt signaling pathway might be used as a therapeutic target for designing new treatment regimens. By targeting Wnt signaling pathway [51, 52, 61] together with NF-κB signaling [21, 31, 58], it is theoretically feasible to design highly effective therapeutic agents for the treatment of HCC and for chemoprevention of hepatocarcinogenesis [66]. The biological significance of the association of *Wnt-1* with NF-κB signaling complexes, the relationship between NF-κB activation and *Wnt-1* protein overexpression, and the activation of *Wnt-1* downstream targets in HBV- and HCV-related hepatocarcinogenesis are important issues remaining to be determined. These are currently being investigated in our laboratories.

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