EUROPEAN JOURNAL OF CANCER 44 (2008) 600–608

available at www.sciencedirect.com

journal homepage: www.ejconline.com

Wilms' tumour 1 gene expression is increased in hepatocellular carcinoma and associated with poor prognosis

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ARTICLE INFO

Article history:
Received 31 October 2007
Received in revised form
21 December 2007
Accepted 7 January 2008
Available online 5 February 2008

Keywords:
Wilms' tumour 1 gene
Hepatocellular carcinoma
Oncogenic potential
Prognosis

ABSTRACT

Background/aims: Wilms' tumour 1 gene (WT1) was originally isolated as a tumour-suppressor gene. We investigated the expression of WT1 in hepatocellular carcinoma (HCC; T) and in non-cancerous hepatic tissues (non-tumour: NT) from patients with chronic liver diseases, and then examined the role of WT1 in the carcinogenesis or prognosis of HCC.
Methods: The expression of WT1 in T and NT from 50 patients with HCC was investigated using Western blotting, immunohistochemistry and real-time reverse transcriptase-polymerase chain reaction (RT-PCR). We also examined whether WT1 expression was related to clinicopathological factors in individual patients in addition to prognostic factors in 50 patients with HCC and in 26 without HCC.
Results: Western blotting and immunohistochemical staining showed that WT1 was overexpressed in T compared with NT ($P < 0.001$) and real-time RT-PCR showed that WT1 mRNA expression was similarly increased. Overexpressed WT1 in HCC was significantly associated with T factors at the TNM stage, and short doubling time of HCC. Univariate and multivariate analyses revealed that WT1 overexpression was an independent prognostic factor for HCC. The disease-free survival period in patients with overexpressed WT1 in NT tissues was significantly reduced.
Conclusion: The expression of WT1 is increased more in HCC than in non-tumour tissues. Moreover, overexpressed WT1 was associated with tumour growth, and resulted in a worsening prognosis of HCC. Our findings from NT tissues revealed that WT1 overexpression might contribute to oncogenic potential.

1. Introduction

Wilms' tumour 1 gene (WT1) that was originally associated with Wilms' tumour[1] is located at chromosome band 11p13 and encodes a transcription factor with four DNA-binding zinc fingers at the C terminus.[2] The gene regulates the transcription of several genes encoding growth factors and growth factor receptors, PDGF-A chain, IGF-II and IGF-IR by citing a few[3–5] as well as other genes (c-myc, bcl-2).[6] In normal human tissue, WT1 expression is restricted to the kidney, testis, ovary, spleen, haematopoietic precursors and mesothelial cell lining of visceral organs.[6] The multifunctional roles of WT1 include activation or repression of transcription, nuclear transcription or RNA metabolism and translational regulation

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.01.008

in the cytoplasm, as well as oncogenic or tumour suppressor functions.[7] Several recent studies suggest that overexpressed WT1 plays an oncogenic, rather than a tumour suppressor role in many types of neoplasm. The wild-type WT1 gene is overexpressed in leukaemia,[8] and in various solid tumours.[9–12] The growth of cancer cells expressing WT1 is inhibited by WT1 anti-sense oligomers.[13] Inoue and colleagues reported that leukaemia with high levels of WT1 mRNA expression resulted in a significantly lower complete remission (CR) rate, and a significantly poorer survival than that with low WT1 expression.[14] High WT1 expression levels in breast cancer are associated with a poor prognosis.[15]

Foetal,[16] but not normal mature liver cells express WT1,[17] yet WT1 is expressed in chronic liver diseases and is associated with their progression.[18] The gene might also play a role in the development of hepatic insufficiency in liver cirrhosis, but its role in hepatocellular carcinoma (HCC), especially how WT1 contributes to its prognosis, remains controversial.

We therefore initially compared the expression and levels of WT1 in HCC and in non-cancerous hepatic tissues from the same individuals, as well as several clinical parameters associated with the prognosis of HCC.

2. Materials and methods

2.1. Patients and liver specimens

Liver specimens of HCC and corresponding non-cancerous hepatic tissues (NT) were obtained from 50 patients who underwent surgery. Table 1a shows the clinicopathological features of these patients. Fresh liver samples were frozen and stored at −80 °C. We extracted proteins for Western blotting from 45 HCC and 46 NT specimens obtained from 50 patients with HCC. We also extracted RNA from 28 HCC and 38 NT specimens for real-time RT-PCR. Extracts from 40 HCC and 46 NT specimens were analysed by immunohistochemistry. Additionally, NT specimens for WT1 immunohistochemical analysis were obtained from 26 patients with chronic liver injury and uncomplicated HCC who underwent surgery or biopsy. Table 1b shows the clinicopathological features of these patients. We diagnosed the patients with complicated, or with uncomplicated HCC based on the findings of angiography or abdominal CT. We finally diagnosed complicated HCC by histological means after surgical resection. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. RNA extraction, cDNA synthesis and real-time RT-PCR

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA was reverse transcribed using RT-PCR kits (Applied Biosystems, Foster City, CA, USA) with an oligo $d(T)_{16}$ primer under standard conditions. Real-time WT1 PCR amplification proceeded using a LightCycler (Roche, Basel, Switzerland) with 2 μl of purified cDNA product, 10 pM of sense primer (5′-GGCATATGAGACCAGTGAGAA-3′, nt 1339–1359), 10 pM of antisense primer (5′-GAGAGTCAGACTTGAAAGCAGT-3′, nt 1821–1800),[14] 1 μl of LightCycler Fast Start DNA Master SYBR

Table 1 – Clinicopathological parameters of 50 patients with HCC (a) and 26 with uncomplicated HCC and chronic liver injury (b)

Clinicopathological parameters variables	*n*, range All patients (*n* = 50)
(a) Complicated HCC	
Age	49 (36–79)
Gender	
Male	40
Female	10
Virus	
HBV	13
HCV	29
Both	0
None	8
Child-Pugh (A/B/C)	45/4/1
Fibrosis	
F1	4
F2	9
F3	12
F4	25
Pathological stage (*n* = 45)	
1	8
2	19
3	12
4	6
AFP	62 (0–7720)
PIVKA-II	81.5 (11–418,820)
PT	90.8 (35.3–180.9)
Tumour size (cm)	
<5	44
>5	6
Tumour differentiation (*n* = 45)	
Poor	5
Moderate	29
Well	11
Tumour multiplicity	
Solitary	40
Multiple	10
Doubling time (d, *n* = 23)	41.2 (4–2921)
Vascular invasion (*n* = 46)	
Absent	36
Present	10
	All patients (*n* = 26)
(b) Uncomplicated HCC	
Age	56 (34–71)
Gender	
Male	20
Female	6
Virus	
HBV	4
HCV	19
Both	0
None	3
Child-Pugh (A/B/C)	24/1/1
Fibrosis	
F1	3
F2	4
F3	9
F4	10
AFP	9.3 (2–427)

HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; PIVKAII, protein induced by vitamin K absence; PT, prothrombin time.

Green I (Roche) and 4 μl of $MgCl_2$ under the following conditions: 40 cycles of 95 °C for 10 s, 62 °C for 10 s and 72 °C for 15 s. This procedure should result in a 482-bp product. Standard curves for WT1 quantitation were constructed from the results of simultaneous amplifications of serial dilutions of the cDNA from HepG2 hepatoblastoma cells that constitutively express WT1.[18]

Glyceraldehyde phosphate dehydrogenase (GAPDH) served as the housekeeping control to determine the relative expression of WT1 mRNA between T (tumour) and NT (non-tumour) tissues. To generate GAPDH mRNA, cDNA was synthesised by RT using an oligo $d(T)_{16}$ primer under standard conditions. We used a commercial GAPDH primer set (Roche Search LC, Heidelberg, Germany) for PCR amplification under the conditions recommended by the manufacturer. The levels of WT1 gene expression divided by those of GAPDH gene expression were defined as relative WT1 mRNA expression levels in the samples.

2.3. *Western blotting*

Liver samples were separated into NT and T tissues, and homogenised with RIPA buffer comprising 10 mmol/L Tris, pH 7.4, 150 mmol/L NaCl 0.5% v/v NP-40 and 1% w/v sodium dodecyl sulphate (SDS). Protein concentrations in lysates were measured using the DC protein assay kit (Bio-Rad, Hercules, CA, USA), and 40 μg of protein was applied to lanes in 4–12% Bis–Tris Gels (Invitrogen). Resolved products were then blotted onto Immunobilon-P membranes (Millipore, Bedford, MA, USA) and incubated with the relevant antibody. Proteins were detected using the ECL Plus Kit (Amersham Pharmacia, Buckinghamshire, UK). The N-terminal monoclonal mouse anti-human WT1 antibody, clone 6F-H2, was obtained from Dako (Tokyo, Japan). A monoclonal antibody to β-actin was obtained from Chemicon (Temecula, CA, USA). Appropriate species-specific conjugated secondary antibodies were obtained from commercial kits (Amersham Pharmacia, Buckinghamshire, UK). The signal intensity of each band on scanned autoradiograms was analysed using Scion Image (Beta 4.0.2, Scion Corporation, Frederick, MD, USA). We normalised each sample to the intensity of the actin band and to that of a positive control (HepG2 cells) band on each film, and then estimated the value as an expression index.

2.4. *Immunohistochemical staining*

Histological diagnoses of tumourous and non-tumourous formalin-fixed and paraffin-embedded tissues were confirmed on haematoxylin and eosin-stained sections. Sections (3 μm thick) were dewaxed, rehydrated and then antigen was retrieved by autoclaving for 1 min at 125 °C in EDTA buffer (pH 9.0). The sections were immersed in methanol containing 0.3% hydrogen peroxidase for 20 min to block endogenous peroxidase activity and then incubated in 2.5% blocking serum for 30 min to reduce nonspecific binding. Primary anti-WT1 monoclonal antibody (Dako) was diluted 1:50 and incubated with the sections at 4 °C overnight.

Stained sections were evaluated in a blinded manner without prior knowledge of the clinical information using the German immunoreactive score (IRS).[19] Briefly, the IRS assigns sub-scores for immunoreactive distribution (0–4) and intensity (0–3), then multiplies them to yield the IRS score (Fig. 1a). We also assessed protein distribution and expression (Fig. 1b).

2.5. *Tumour doubling time*

We determined the tumour-doubling time using preoperative CT or MRI and calculated tumour volume as described.[20] We then calculated tumour growth rates using the formula developed by Schwartz.[21] Of the patients who underwent hepatic resection, we did not include those who underwent preoperative transarterial chemoembolisation, percutaneous ethanol injection, or CT or MRI only once before hepatic resection, because we could not estimate the natural tumour growth.

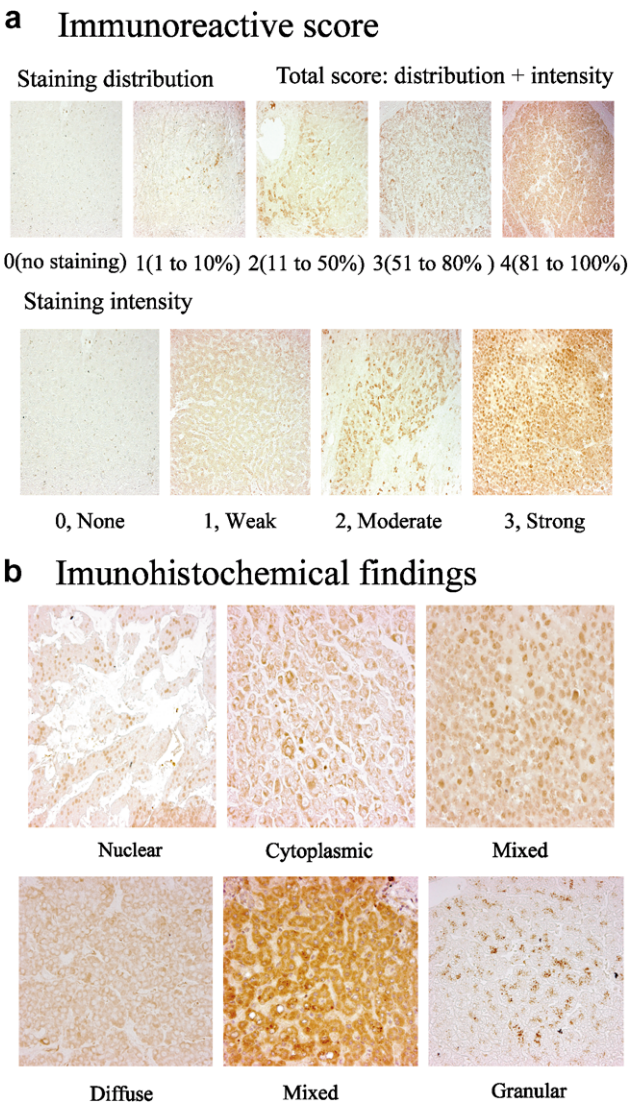

Fig. 1 – (a) Typical scored immunohistochemical staining. Total score was calculated from sub-scores of staining distribution (0–4) and intensity (0–3). (b) Immunohistochemical staining findings.

2.6. Statistical analysis

All statistical analyses were performed using SPSS 14.0 software (SPSS, Chicago, IL, USA). Disease-free survival rates were calculated using the Kaplan–Meier method, and differences were assessed using the log rank test. The patient cohort was sufficiently large to allow statistical analysis of disease-free survival. The relative influence of various prognostic factors was evaluated using Cox multivariate proportional hazards regression analyses. Significance was established at $P < 0.05$.

3. Results

3.1. Expression of WT1 is increased in hepatocellular carcinoma

Expression of WT1 and β-actin was assessed by Western blotting for 45 HCC and 46 non-cancerous hepatic samples from patients with HCC (Fig. 2a). We normalised the intensity of each protein band against the expression of β-actin in each sample, and calculated this ratio as an expression index. We used human lung and kidney tissues as positive controls. WT1 protein was significantly overexpressed in HCC tissues (tumour; T) compared with paired surrounding non-tumour tissue (NT) (2.03-fold, $P < 0.001$) (Fig. 2b). We detected WT1 in all T samples, and in 87% of NT samples.

Immunohistochemical staining of NT and T tissues for WT1 revealed that positive WT1 signals localised to the nucleus and cytoplasm of hepatocytes. We detected WT1 protein in 95% of T (38/40 samples), and in 63% of NT (29/46 samples). We sub-scored the staining distribution and intensity, and then compared the total score between T and NT (Fig. 2c). Significantly, more WT1 protein was expressed in T than in NT (4.30 ± 2.01 versus 1.98 ± 1.76, $P < 0.001$). Moreover, the distribution and intensity significantly correlated in both T and NT of HCC according to Spearman's rank analysis ($P < 0.001$). We detected WT1 protein mainly in the cytoplasm compared with the nucleus (nucleus 5 (13%), mixed 4 (11%), cytoplasm 29 (76%) of 38 samples). WT1 staining was granular in 16 (42%), and diffuse in 22 (58%) samples. Interestingly, granular staining was predominant in hepatitis C virus (HCV)-negative samples ($P = 0.033$; Fisher's exact test).

We performed real-time RT-PCR for WT1 mRNA from frozen paired samples derived from patients with HCC. We calculated the copy number ratio of WT1 mRNA/GAPDH mRNA (Fig. 2d). This ratio was significantly higher in T than in NT tissues (7.45×10^3 versus 1.68×10^3; median copy number, $P = 0.027$). All T samples were positive for WT1 mRNA, but 2 NT samples were negative (5% of NT samples). In addition to WT1 protein, WT1 transcription was enhanced in HCC rather than in the surrounding cirrhotic tissue.

3.2. Relationship between WT1 protein expression and clinical features of HCC

We analysed the relationship between WT1 protein expression and clinical features of HCC by determining the expression index of T and NT in each patient, calculating the T/NT ratio and then establishing groups with high and low WT1 expression based on the results of Western blotting. Fig. 3a shows that by using a cutoff level of 1.63 (median value of the T/NT ratio), 21 patients each were categorised into groups expressing high and low levels of WT1. We compared the clinicopathological features between the two groups and summarised the findings in Table 2.

Tumour factor in the TNM classification (indicating tumour size and multiplicity) was significantly higher in the high, than in the low WT1 group ($P = 0.026$). Moreover, more

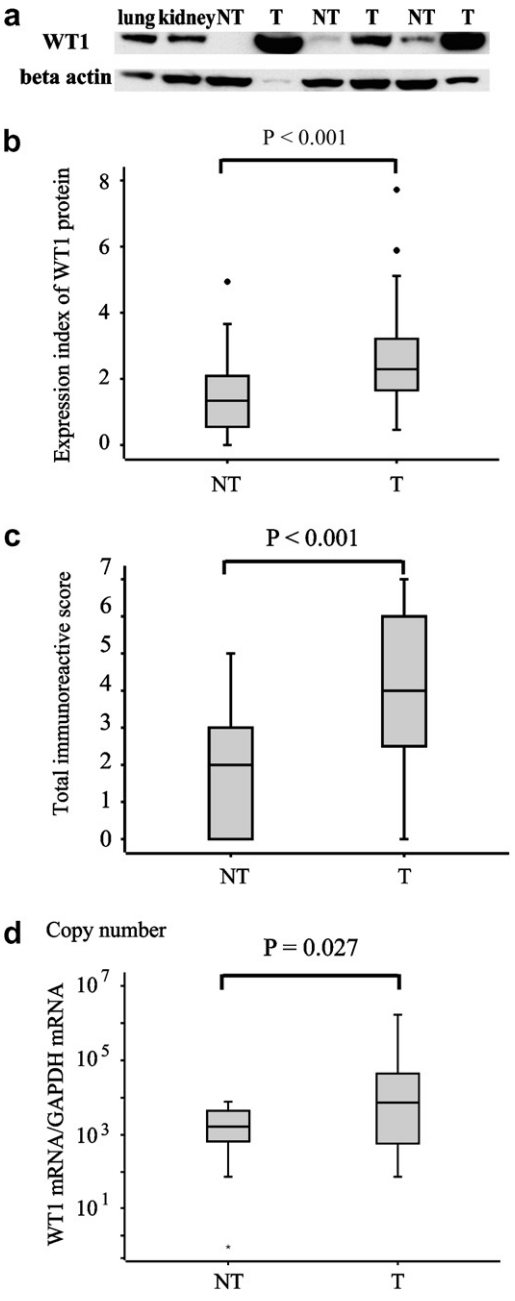

Fig. 2 – Level of WT1 expression in HCC (T) and in non-cancerous hepatic tissues (NT). (a) Expression of WT1 was assessed by Western blotting. (b) WT1 protein was significantly overexpressed in T. (c) Immunohistochemical detection of WT1. (d) Level of WT1 mRNA.

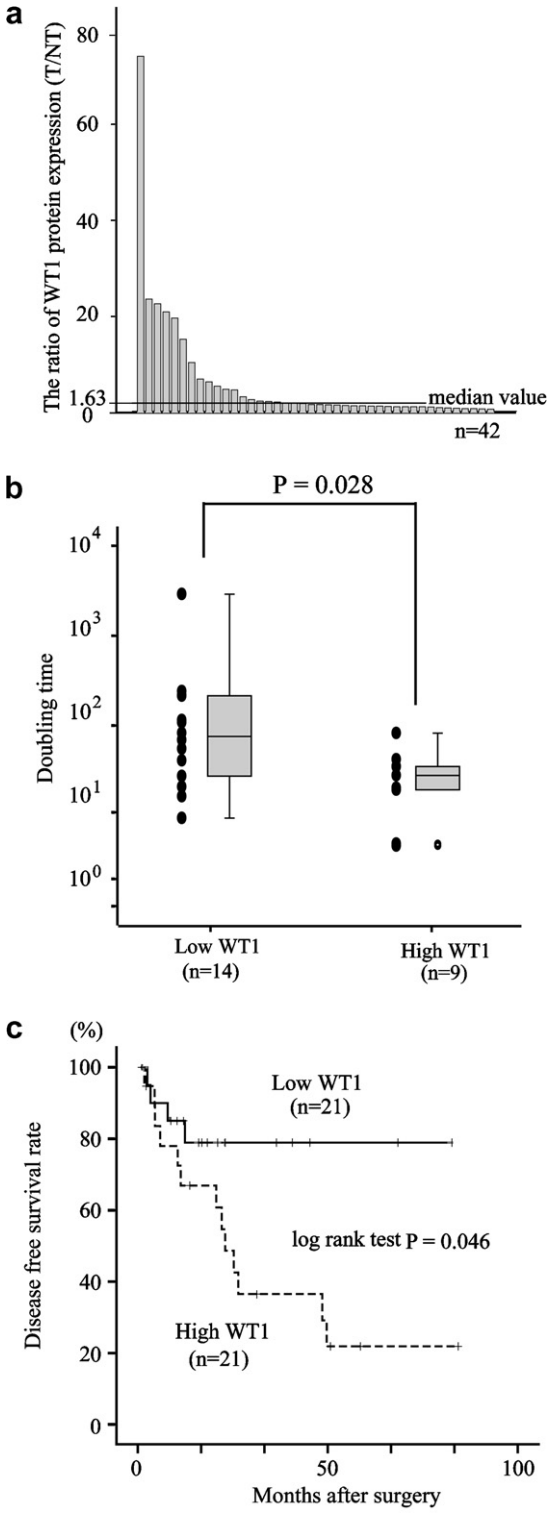

Fig. 3 – (a) Patients were assigned to high and low WT1 groups for statistical analysis using a cutoff value of 1.63. (b) Relationship between WT1 protein expression and HCC doubling time. (c) Kaplan–Meier plot of disease-free survival rates of HCC patients Abbrev.: T = tumour tissue; NT = non-cancerous hepatic tissue.

HCC patients had a shorter doubling time (<100 d) in the high, than in the low WT1 group according to the χ^2-test ($P = 0.030$). Fig. 3b shows that the doubling time was shorter in the high, than in the low WT1 group (Mann–Whitney *U*-test). WT1 expression was more frequently high in AFP-L3 positive patients with weak statistical significance (Table 2; $P = 0.076$). The expression of WT1 did not significantly correlate with any other variables including tumour differentiation.

We analysed disease-free survival rates of the two groups to assess the prognostic significance of WT1 expression. Patients who expressed higher WT1 levels had shorter 5-year disease-free survival rates from recurrence than those with lower WT1 expression (22% versus 79%, $P = 0.046$; Fig. 3c). To assess the clinical importance of WT1 expression in HCC, we performed multivariable Cox regression analysis to identify factors that might predict disease-free survival after hepatectomy. Table 3 shows that a high level of WT1 expression was significantly associated with disease-free survival (relative risk, 5.78; $P = 0.013$). Pathological stage, tumour multiplicity and vascular invasion were also independent prognostic factors of disease-free survival.

3.3. *Clinicopathological role of WT1 expression in non-cancerous hepatic tissues*

To analyse the clinicopathological role of WT1 expression in non-cancerous hepatic tissues, we analysed 26 other non-cancerous hepatic tissues obtained from patients with uncomplicated HCC by liver biopsy or during surgery for other liver diseases. Table 1b describes the clinical background of the patients from whom these samples were obtained.

Immunohistochemical staining detected positive WT1 signals in non-cancerous hepatic tissues from 38 of 72 patients (53%), the prevalence of which was associated with the progression of liver fibrosis (Fig. 4a). These were similar to previous findings.[18] Here, we located WT1 protein in the cytoplasm of hepatocytes from both non-cancerous and HCC tissues. However, no samples were positive for WT1 in the nucleus (nuclear 0/38 (0%), mixed 6/38 (16%), cytoplasmic 32/38 (84%)). Interestingly, granular WT1 protein distribution was predominant in HBs-Ag-positive patients ($P = 0.015$, Fisher's exact test). However, diffuse WT1 protein distribution predominated in HCV-positive patients ($P = 0.001$, Fisher's exact test).

Fig. 4b shows that the immunohistochemical staining score was higher in the non-cancerous tissues from patients with complicated, than uncomplicated HCC (2.0 ± 1.75 versus 0.58 ± 0.99, $P < 0.001$). Because a proportional increase in WT1 protein was related to the progression of liver fibrosis (Fig. 4a), we re-evaluated our findings in liver tissues only from patients with stage 3 and 4 fibrosis (Fig. 4c).[22] The results were similar to those shown in Fig. 4b. The score was higher in non-cancerous tissue from complicated, than from uncomplicated HCC (2.06 ± 1.58 versus 0.63 ± 0.96, $P = 0.001$). These results indicated that the potential of liver tissue to develop hepatocarcinogenesis is higher when WT1 is overexpressed.

Based on the immunohistochemical data of non-cancerous tissues, we separated samples from 43 non-cancerous liver tissues of HCC patients who underwent successful surgical resection into low (0–2 immunohistochemical staining score) and high (3–7 immunohistochemical staining score)

Table 2 – Correlation between WT1 expression and clinicopathological parameters in 42 patients with complicated HCC

Variables	All patients ($n = 42$)		P value
Clinicopathological parameters	High WT1 ($n = 21$)	Low WT1 ($n = 21$)	
Age			
<65 years	13	10	
>65 years	8	11	0.268[a]
Gender			
Male	17	18	
Female	4	3	0.500[a]
Virus			
HBV	5	6	
HCV	13	13	
None	3	2	0.865[b]
Child-Pugh			
A	19	20	
B	2	1	
C	0	0	0.500[a]
Pathological stage			
1/2	10	15	
3/4	11	6	0.104[a]
T factor			
1/2	10	17	
3/4	11	4	0.026[a]
JIS score			
0/1	10	15	
2/3/4	11	6	0.104[a]
AFP			
>100 ng/ml	7	6	
<100 ng/ml	14	15	0.500[a]
AFP-L3			
Positive	10	4	
Negative	11	15	
Not done	0	2	0.076[a]
PIVKAII			
<40	5	6	
>40	14	15	
Not done	2	0	0.578[a]
Tumour size			
<3 cm	8	12	
>3 cm	13	9	0.177[a]
Tumour differentiation			
Poor	2	2	
Moderate	12	14	
Well	5	5	
Unknown	2	0	0.973[b]
Growth status			
Expansive growth	15	13	
Invasive growth	4	8	
Unknown	2	0	0.204[a]
Tumour multiplicity			
Solitary	16	17	
Multiple	5	4	0.500[a]
Doubling time (d, $n = 23$)			
>100 d	0	6	
<100 d	9	8	0.030[a]
Formation of fibrous capsule			
Present	15	13	
Absent	5	8	
Unknown	1	0	0.287[a]
Vascular invasion			
Present	5	4	
Absent	15	17	
Unknown	1	0	0.466[a]

Table 2 – (*continued*)

High and low WT1: <1.63 WT1/beta actin and >1.63 WT1/beta actin, respectively, in T divided by WT1/beta actin in NT.
a – Fisher's exact test.
b – χ^2-test. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, Lens culinaris agglutinin A-reactive fraction of alfa-fetoprotein; PIVKAII, protein induced by vitamin K absence; T factor, tumour factor; JIS score, Japan Integrated Staging score.

Table 3 – Multivariable Cox regression analysis of disease-free survival

	Odd ratio	95% Confidence interval	P value
WT1 expression (low: high)	5.78	1.450–23.068	0.013
Pathological stage (1 and 2; 3 and 4)	11.8	1.089–126.95	0.042
Tumour multiplicity (solitary: multiple)	12.6	1.279–124.30	0.030
Vascular invasion (absent: present)	15.5	1.864–128.72	0.011

Variables: WT1 expression (low versus high), pathological stage (1 and 2 versus 3 and 4), tumour multiplicity (solitary versus multiple), vascular invasion (absent versus present), AFP (<100 versus >100 ng/ml), tumour size (<5 cm versus >5 cm), liver cirrhosis (absent versus present).

WT1 groups. We analysed the relationship between WT1 expression levels in non-cancerous surrounding tissue and the clinical prognosis of HCC patients using the Kaplan–Meier method (Fig. 4d). Disease-free-survival rates were significantly lower in the high, than in the low WT1 group (26% versus 40%, $P = 0.042$). Overall, non-cancerous liver tissue might have a high risk of HCC occurrence as well as HCC recurrence among patients with chronic liver diseases.

4. Discussion

This is the first study to show that WT1 protein and RNA are overexpressed in hepatocellular carcinoma (HCC) compared with the surrounding non-cancerous tissues. Moreover, high WT1 expression in HCC was significantly associated with doubling time, as well as with the pathological T factor of TNM stage classification. Because tumour-doubling time is recognised as a significant prognostic factor,[23] the short doubling time of HCC and high T score of HCC overexpressing WT1 might relate to a poor prognosis of HCC. This has been found in leukaemia,[14] and we also showed that increased WT1 expression was associated with the occurrence of HCC in non-cancerous tissues with or without HCC. Disease-free-survival rates after curative HCC resection were significantly lower in the WT1 group with a high, than with a low expression score, indicating that WT1 overexpression in non-cancerous hepatic tissue is also related to a poor prognosis due to the early recurrence of HCC. These results indicated that the level of WT1 expression is associated with the malignant potential of HCC, with the potential for oncogenesis or

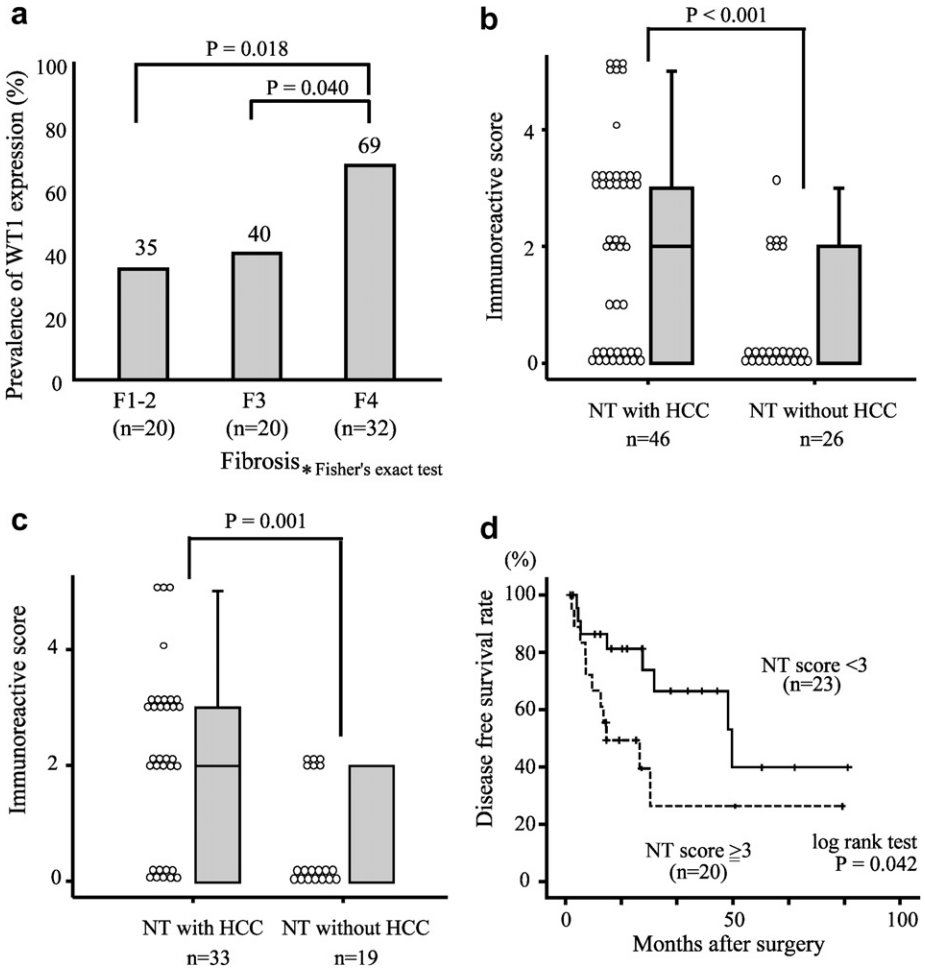

Fig. 4 – Analysis of immunoreactive score in NT. (a) Ratio of WT1 expression prevalence in chronic liver injury. (b) Immunoreactive score in NT with or without HCC. (c) Scores from patients with fibrosis stages 3–4. (d) Disease-free survival rates according to WT1 expression. Abbrev.: NT = non-cancerous hepatic tissue; *F* = fibrosis stage; HCC = hepatocellular carcinoma.

re-carcinogenesis in surrounding tissues, and it is also linked to the prognosis of HCC.

The WT1 gene encodes a transcription factor that is important for normal cellular development and cell survival in hepatocytes from the foetal, but not from the mature liver. However, Berasain and colleagues reported that WT1 is expressed in the cirrhotic liver in relation to disease progression.[18] They found that WT1 is induced by transforming growth factor beta (TGF-β) and down-regulated by hepatocyte nuclear factor 4 (HNF-4) in liver cells. Lazarevich and colleagues reported that a loss of HNF-4 expression is an important determinant of HCC progression.[24] Considering these findings, overexpressed WT1 might be a consequence of HNF-4 down-regulation and thus leads to a poor prognosis of HCC. In contrast, Idelman and colleagues reported that the transcriptional activity of WT1 proteins and their ability to function as tumour suppressors or oncogenes depends on the cellular status of p53,[25] which modulates the ability of WT1 to bind to the insulin-like growth factor-I receptor (IGF-IR) that plays a pivotal role in tumourigenesis.[25] Interaction between these proteins might contribute to overexpressed WT1 in HCC or to the tumourigenesis of WT1. Further investigations are required to clarify these notions.

WT1 protein is regarded as a transcription factor that is localised to the nucleus. However, WT1 protein is mainly detected, for example, in the cytoplasm of pancreatic cancer, soft tissue sarcoma, osteosarcoma, glioblastoma and malignant melanomas of the skin.[26] Niksic and colleagues reported that 10–50% of the total cellular WT1 can be detected in the cytoplasm depending on the cell type.[27] Our staining studies also showed that WT1 protein resided mainly in the cytoplasm of HCC (76% in total). We also found that granular staining was significantly more frequent in non-cancerous tissues from HBs-Ag positive patients, and significantly less so in non-cancerous tissue from HCV-positive patients. WT1 staining was frequently granular in various tumour tissues,[28] which is due to localization in organelles, granules or secretory vesicles.[29] These results indicate that WT1 associates with viral particles or with cellular proteins modulated by these viruses.

We speculate that overexpressed WT1 plays critical roles not only in the cell growth of HCC, but also in its carcinogenesis or prognosis. Moreover, WT1 was associated with the

stage of fibrosis in chronic liver disease. The present study showed that a high level of WT1 expression in non-cancerous tissues is associated with a higher incidence of HCC, suggesting that WT1 protein has oncogenic activity in chronic liver diseases. Identifying the expression level of WT1 by immunohistological staining would have clinical value in terms of estimating the doubling time of HCC and its prognosis in cancerous tissue, and the risk of HCC occurrence in non-cancerous tissue. Recently, WT1 has been targeted for immunotherapy of various cancers and sarcomas.[30] Here, we showed that WT1 is overexpressed in HCC, especially those with a poor prognosis. The present findings suggest that WT1 expression is not only a clinical indicator of a poor prognosis but it also has potential as a target for immunotherapy against HCC.

Conflict of interest statement

None declared.

Acknowledgements

This study was supported by Grant-in-Aid for Scientific Research (JSPS KAKENHI 19590771) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (to Y.H.).

We thank Mr. Kenji Tanimoto, Ms. Satomi Yamanaka and Ms. Chie Matsugi for valuable contributions to this study.

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