P21/WAF1 is an independent survival prognostic factor for patients with hepatocellular carcinoma after resection

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Keywords

Abstract

Background/Aims: The cyclin kinase inhibitor p21/WAF1 is regulated by p53-dependent or independent pathways and inhibits the action of proliferating cell nuclear antigen (PCNA). The prognostic role of p21/WAF1 in hepatocellular carcinoma (HCC) is ambiguous. To further clarify this, we examined the expression of three genes in HCC. Methods: A total of 122 resected HCC specimens were collected from 1987 to 1998. Expression of p21/WAF1, p53, and PCNA in HCC was analysed by immunohistochemistry. Results: Immunoreactivity was detectable for p21/WAF1 in 37%, and for p53 in 41.8% of HCCs. Positive expression of both genes does not relate to each other, but both are associated with a high PCNA labelling index (LI) (\(P < 0.05\)) in tumour. p53 (+) is also associated with high serum \(\alpha\)-foetoprotein (\(\alpha\)FP) (\(P < 0.001\)), tumour dedifferentiation (\(P = 0.001\)) and advanced pathologic stages (\(P = 0.017\)). However, p21/WAF1 (+) did not show clinicopathologic significance. Survival analysis indicated that poor prognostic factors were p21/WAF1 \((-)\) (\(P = 0.024\)), p53 \((+)\) (\(P = 0.008\)), high PCNA (\(P < 0.001\)), tumour without capsule (\(P = 0.001\)), poor tumour differentiation (\(P = 0.004\)), advanced pathologic stage (\(P < 0.001\)), and high serum \(\alpha\)FP (\(P < 0.001\)). Independent factors were p21/WAF1 expression, pathologic stage, and PCNA. Conclusion: In HCC, increased proliferation index PCNA is significantly associated with positive p53 and p21/WAF1. But p21/WAF1 expression did not relate to p53 expression. P21/WAF1 (+) is a good event and serves as an independent survival prognostic factor for HCC, which is a novel finding apart from previous reports.

Hepatocarcinogenesis is considered to be a multifactorial and multistep process involving different genetic alterations that ultimately lead to malignant transformation of the hepatocyte. Investigation has demonstrated chromosomal allelic losses in hepatocellular carcinoma (HCC) tissues, suggesting the deletion or alteration of tumour suppressor genes, which may play a role in the development and progression of HCC (1). In HCC, loss of heterozygosity (LOH) has been reported on chromosomes 1p, 4q, 5q, 8p, 10q, 11p, 13q, 16, 17p, and 22q (1). At present, only a few tumour suppressor genes located in these deleted regions have been clearly involved in a significant subset of HCC, like the p53 gene (17q13) (2, 3), the Rb gene (13q14) (4), and the APC gene (5q21) (5). Among these, the p53 gene is the gene most widely studied. The human p53 tumour-suppressor gene, as a transcription factor, plays an important role in the regulation of the cell cycle, maintenance of genomic stability, cell differentiation, and apoptosis (6). The p53 gene is mutated in about 18–67% of HCC worldwide (2, 7), and plays an important role in the genesis or progression of HCC. The prognostic role of structural abnormality in the p53 gene for HCC has also been deciphered previously (8). Further, the p53 gene also regulates the expression of proliferating cell nuclear antigen (PCNA) in HCC (9, 10). PCNA is a nuclear protein, which is closely related to the cell cycle regulation being an auxiliary molecule for DNA polymerase-\(\delta\) (11). PCNA can be applied as a useful marker for detecting proliferating cells and correlated to histologic characteristics and prognosis in HCC (9, 10). In HCCs, high PCNA values were found to affect prognosis adversely.
Numbers of down-stream genes containing wild-type p53 binding sequences have been identified. Among these, p21/WAF1 is the most important one. p21/WAF1 is a nuclear protein and induces cell cycle arrest in the G1 and G2 phases by inhibiting cyclin/cyclin-dependent kinase (CDK) complexes and PCNA function (12). PCNA and p21/WAF1 actively interact in the response to DNA damage. After ultraviolet (UV) challenge, p21/WAF1 and PCNA colocalize in the nucleus and become detergent insoluble, owing to a tight link to DNA (13). P21/WAF1 can be activated in p53-dependent and -independent manners (14). Cells lacking functional p53 express a very low level of p21/WAF1 and the p21/WAF1 promoter contains a p53-binding site, suggesting that expression of p21/WAF1 depends on p53 function (6, 14, 15). However, p21/WAF1 can be inducible in p53-null cells, showing that the expression of p21/WAF1 can also be induced by p53-independent pathways (14, 16, 17).

Previous reports have suggested that p21/WAF1 mRNA expression in non-tumour liver tissues is significantly higher than that in HCC tissues (18, 19). These results indicate that p21/WAF1 expression might represent a form of CDK inhibitor dysfunction involved in tumourigenesity (19, 20). Furthermore, it has been demonstrated that p21/WAF1 expression might be influenced by viral proteins in human chronic liver disease (21). However, the prognostic role of p21/WAF1, the relationship between p21/WAF1 expression and carcinogenesis in chronic liver disease has not been well elucidated and has been controversial (20, 22–25), although p21/WAF1 is upregulated and related to proliferation in human liver diseases (26). Further, there were rare reports dealing with the linkage of three genes of p21/WAF1, p53 and PCNA in HCC in which the results are different (25, 27, 28).

In the present study, we examine an immunohistochemical technique to investigate the possible role of p21/WAF1 based on a relatively large sample size of 122 human HCCs after resection and elucidate the correlation of clinicopathological features between p21/WAF1 and p53, PCNA. Finally, we evaluate the poor prognostic factors for disease-free survival and disease-specific survival analysis in HCC patients with at least 7 years follow-up. We found that patients with a positive expression of p21/WAF1 in HCC had a longer disease-specific survival after resection, which is a novel finding.

Materials and methods

Resected HCC specimens

A total of 122 HCC paraffin specimens were collected by surgical resection at Department of Pathology at Kaohsiung Chang Gung Memorial Hospital from January 1987 to December 1998. All the HCC patients were diagnosed with resectable tumour(s) after liver biochemical test, and complete imaging studies like sonography, computed tomography, and/or angiography. Patients who died soon owing to postsurgical complication and patients who were lost to follow-up were excluded from this study. The closing date of follow-up was 31 December 2006. The durations of follow-up were estimated in months. The survival of patients who died owing to HCC-unrelated factors during the long periods of follow-up was estimated till the event and treated as censored data. Hepatitis markers, serum α-fetoprotein (αFP) levels, and other clinical parameters of HCC patients were also recognized. All the HCC specimens consisted of both tumour and adjacent non-tumour parts. Tumour sizes were recorded as the largest diameter in the specimen. The background of the non-tumour part was characterized as cirrhotic or non-cirrhotic. The differentiated states of HCC were divided into three groups as well (grade I carcinoma of Edmondson–Steiner classification), moderate (grade II carcinoma of Edmondson–Steiner classification), and poor (grades III and IV carcinoma of Edmondson–Steiner classification). The pathologic stages of HCC were classified according to the staging system by the International Union against Cancer with a minor modification: stage I, encapsulated, without evidence of liver or vascular invasion; stage II, unencapsulated or capsulated and with liver invasion, but without vascular invasion; stage III, invasion of small vessels in the tumour capsule or focal invasion of portal vein branches close to the tumour; and stage IV, invasion of portal vein in the distal liver (1 cm away from the tumour capsule), branches of major portal vein, common bile duct, or perforation into visceral peritoneum.

Immunohistochemistry

The paraffin sections from HCC specimens were deparaffinized, blocked with 3% hydrogen peroxide for 10 min, and subjected to antigen retrieval with a microwave in 0.01 M citrate buffer for 15 min. The slides were then washed twice with PBS, incubated with p21/WAF1 antibodies (1:50 dilution; Zymed, San Francisco, CA, USA), p53 antibodies (1:100 dilution; Biogenex, San Ramon, CA, USA), and PCNA antibodies (1:100; Novocastra, Newcastle, upon Tyne, UK) for 30 min, and then detected with peroxidase conjugate using a polymer detection system (Zymed Cat. No. 87-89431) for 30 min. The antibody staining was visualized with 3,3-diaminobenzidine...
tetrahydrochloride (DAB; Sigma, St. Louis, MO, USA) in 0.1 M Tris pH 7.2, containing 0.01% H₂O₂. The section slides were counterstained with Gill’s haematoxylin, dehydrated, and mounted.

**Immunohistochemical scoring**

The percentages of immunostaining on clinical samples were expressed as a labelling index (LI) by a pathologist. Normal oesophageal squamous epithelium and HCC with known p53 protein overexpression were used as positive controls for p21/WAF1 and p53 respectively. Negative controls were obtained by omitting primary antibody. P21/WAF1 and p53 immunostaining was assessed quantitatively by counting the total number of positively stained nuclei per 10 high-power fields (× 400 magnifications) microscopically from the tumours and non-tumourous liver, respectively. High-power fields were used for including more cells to be counted and to allow better representation because of intratumoural heterogeneity of staining. Only nuclear staining was considered to be positive for p21/WAF1 and p53. Based on the previously published criteria, positive staining of p21/WAF1 was considered when ≥5% of tumour cells were stained (29). Positive scoring for p53 was considered when ≥10% of the nuclei were stained, according to the criteria used previously (30, 31). The LI of nuclear PCNA was assessed as described previously (10). Briefly, the sections were scanned at a low power to determine the areas that were most evenly and heavily labelled. Regardless of staining intensity, the nuclear LI was expressed as the average percentages of cells with PCNA nuclear staining from five different high-power fields (× 400 magnifications). Subsequently, the correlation between three genes and clinicopathologic features of HCC including histological tumour grades (differentiation) and pathologic stages, survival outcome, serum levels of αFP, cirrhotic background, hepatitis markers, and tumour sizes were analysed by statistical analysis.

**Statistical methods**

Comparisons between groups of independent samples were assessed by the Student’s t-test or Mann–Whitney U-test. The associations between categorical variables were assessed using the χ² test or Fisher’s exact test. Survival rates were calculated by the Kaplan–Meier methods, and the difference in survival was compared with the log-rank test. The influence of various clinicopathologic features on overall survival was assessed by Cox’s proportional hazard model. A P-value of < 0.05 was considered to be statistically significant.

**Results**

**Clinical parameters of HCC patients**

The surgically resected specimens were collected from 122 HCC patients consisting of 98 males and 24 females. The ages ranged from 25 to 81 years, with a mean age of 54.4 ± 11.6 years. The overall survival rate was 45.9% (56/122) at the end of at least 7 years of follow-up. Tumour sizes ranged from 1 to 20 cm, with a mean size of 6.24 ± 3.85 cm. The differentiation extents in HCC samples were graded into three categories as well-differentiated HCC (in 28 cases), moderately differentiated HCC (in 60 cases), and poorly differentiated HCC (in 34 cases). The tumour pathologic stages were divided into stage I (in 19 cases), stage II (in 40 cases), stage III (in 37 cases), and stage IV (in 26 cases). In the background of the non-tumour part, liver cirrhosis was found in 75 cases (61.5%) and non-cirrhotic in 47 cases (38.5%). The status of hepatitis B virus (HBV) and hepatitis C virus (HCV) in these HCC patients was examined. The hepatitis B surface antigen (HBsAg) was detected in the sera of 81 patients (66%), and anti-HCV was detected in 29 patients (24%). Five patients (4%) were positive for both HBV and HCV, whereas 10 patients (8%) were negative for both markers. Six patients did not receive any survey of hepatitis B or C infection.

**Expression of p21/WAF1, PCNA, and p53 protein in HCC**

Forty-five of 122 tumours (36.9%) exhibited positive p21/WAF1 nuclear staining. The distribution of positive nuclear p21/WAF1 immunoreactivity is major on a scattered pattern (5–30%) (23/45, 51.1%), followed by a moderate (30–50%) (15/45, 33.3%), and diffuse pattern (> 50%) (7/45, 15.6%) (Fig. 1). In non-tumourous liver, most cases (> 90%) were negative staining for p21/WAF1. Cases with positive immunoreactivity in non-tumour parts always exhibited a scattered staining for p21/WAF1. Subsequently, we analysed p53 and PCNA expression in HCC specimens. Immunohistochemical studies demonstrated that p53 and PCNA staining was sporadically or diffusely localized in the nucleus of HCC tissues (Fig. 2). In 122 HCC specimens, p53 (+) was found in 51 cases (41.8%). In contrast, PCNA was almost positive in HCC with a wide range of LI (2–98%), with a mean of 70.9 ± 24.0% and a median of 79%. From statistics, tumours with either positive p21/WAF1 or
Fig. 1. Immunohistochemical studies of p21/WAF1 in four hepatocellular carcinoma tissues that exhibit different levels of nuclear immunoreactivities to p21/WAF1 from negative, scattered, moderate to diffuse pattern (× 400 magnifications).

Fig. 2. Immunohistochemical studies of p53 and proliferating cell nuclear antigen (PCNA) in two hepatocellular carcinoma (HCC) cases. Case 1 HCC, who was free from tumour recurrence and survived more than 10 years after resection, is negative for p53 expression with only scattered PCNA expression. In contrast, case 2 HCC, who had tumour recurrence 6 months after resection, exhibits abundant p53 and PCNA expression (× 200 magnifications).
p53 exhibited a higher mean PCNA LI (Tables 1 and 2; P = 0.015 for p21, and < 0.001 for p53). When comparing p21/WAF1 expression with p53 accumulation, no significant association was found between p21/WAF1 and p53 expression (P = 0.75) (Tables 1 and 2). We further compare p53 and p21/WAF1 expression with other clinicopathologic parameters of HCC. The relationship between p21/WAF1 expression and the clinicopathologic findings of the HCCs in the 122 cases is summarized in Table 1. All the parameters did not reveal a significant association with difference between these two groups, except PCNA (P = 0.015). In contrast, p53 (+) was associated with many poor prognostic factors of HCC, such as increased grades (P = 0.005), advanced pathologic stages (P = 0.026), high serum αFP levels (P = 0.045), cirrhotic background (P = 0.08), and younger patients (P = 0.08) (Table 2).

### Survival and tumour recurrence analysis

Kaplan–Meier analysis indicated that the disease recurrence and disease-specific survival in HCC patients with p53 (+) were significantly earlier and shorter.
than those of the patients with p53 (−) (Fig. 3C and D; disease recurrence, $P = 0.02$; survival, $P = 0.009$ respectively). However, there was no significant difference in the disease recurrence between the positive and negative p21/WAF1 expression. In contrast, disease-specific survival of the patients with p21/WAF1 (+) was significantly longer than that of patients with p21/WAF1 (−) (Fig. 3A and B; disease recurrence, $P = 0.024$; survival, $P = 0.009$).

Fig. 3. Kaplan–Meier analyses of disease-specific survival and tumour recurrence in hepatocellular carcinoma (HCC) patients after surgery. Positive p21/WAF1 expression in HCC does not relate to tumour recurrence (A), but predicts longer survival of patients after resection (B). In contrast, patients with positive p53 expression had earlier disease recurrence (C) and shorter survival (D) than patients with negative p53 expression. Likewise, patients with high proliferating cell nuclear antigen (PCNA) expression also had earlier disease recurrence (E) and shorter survival (F) than patients with low PCNA expression. $n$, the number of patients in each group.
For survival analysis, PCNA was divided into two groups as high or low by ROC curve. Like p53, the disease recurrence and disease-specific survival of the patients with high PCNA were significantly earlier and shorter than those of the patients with low PCNA (Fig. 3E and F; disease recurrence, \( P < 0.001 \); survival, \( P < 0.001 \) respectively). These results further showed that HCC with p53 (+) and high PCNA expression had an unfavorable prognosis, whereas the presence of p21/WAF1 in HCC is a good event for survival.

**Univariate and multivariate analysis of prognostic factor for HCC**

To evaluate the potential of using these genes for the prognosis of HCC patients after surgery, univariate and multivariate analysis in Cox’s proportional hazard model revealed that p53 (+), high PCNA, advanced pathologic stage, high serum \( \alpha \)-FP, absence of a tumour capsule, increased HCC dedifferentiation, and large tumour size could predict earlier tumour recurrence after resection. Independent factors are PCNA [risk: 2.8; 95% confidence interval (CI): 1.65–4.74; \( P < 0.001 \)], pathologic stage [risk: 2.36; 95% CI: 1.46–3.82; \( P < 0.001 \)], and serum \( \alpha \)-FP [risk: 2.53; 95% CI: 1.59–3.99; \( P < 0.001 \)] (Table 3). In addition, the prognostic factors for disease-specific survival are similar to tumour recurrence (Table 4). Notably, although not related to tumour recurrence, the presence of p21/WAF1 in HCC could predict longer patient’s survival after resection and serve as an independent factor (Table 4). It is a novel finding for this study that was not reported before.

**Discussion**

In the present study, we provide evidence for p21/WAF1 protein expression in 36.9% and p53 expression in 41.8% of HCC samples for the diagnostic value of p21/WAF1 and p53 as prognostic factors for HCC patients after surgery. It is a novel finding for the prognostic role of p21/WAF1, which was not reported before in HCC, but in gastric carcinoma (32). Two tumour suppressor genes were demonstrated to correlate positively with the proliferating states (PCNA) of HCC, which was a well-known prognostic factor for HCC patients.

PCNA is an auxiliary protein present during the G1-late phase and S phase. The increased PCNA LI of HCC closely correlated with the advanced histologic grades, pathologic stages, and poor patients’ outcome (9, 10). P53, a tumour suppressor gene, has an important function in DNA repair and in

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**Table 3. Correlation of clinicopathologic factors and disease recurrence of HCC**

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate</th>
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<tr>
<td></td>
<td>Risk</td>
<td>95% CI</td>
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<tr>
<td><strong>Biomarkers</strong></td>
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<tr>
<td>P21</td>
<td>1.10</td>
<td>0.71–1.70</td>
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<tr>
<td>p53</td>
<td>1.62</td>
<td>1.06–2.47</td>
</tr>
<tr>
<td>PCNA</td>
<td>3.71</td>
<td>2.37–6.05</td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
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<tr>
<td>Age</td>
<td>0.78</td>
<td>0.62–1.52</td>
</tr>
<tr>
<td>Gender</td>
<td>0.82</td>
<td>0.52–1.63</td>
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<tr>
<td>AFP</td>
<td>2.78</td>
<td>1.79–4.31</td>
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<tr>
<td>HBV</td>
<td>1.31</td>
<td>0.88–2.10</td>
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<tr>
<td>HCV</td>
<td>0.62</td>
<td>0.35–1.16</td>
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<tr>
<td>Cirrhosis</td>
<td>1.31</td>
<td>0.69–2.01</td>
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<tr>
<td><strong>Path parameters</strong></td>
<td></td>
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<tr>
<td>Tumour capsule</td>
<td>2.25</td>
<td>1.32–3.65</td>
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<tr>
<td>Tumour size</td>
<td>1.58</td>
<td>1.03–2.43</td>
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<tr>
<td>Tumour number</td>
<td>1.33</td>
<td>0.83–2.29</td>
</tr>
<tr>
<td>Pathologic stages</td>
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<td>2.02–4.95</td>
</tr>
<tr>
<td>Grade</td>
<td>1.48</td>
<td>1.10–2.01</td>
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</tbody>
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P21, positive or negative; p53, positive or negative; PCNA, high or low (by ROC curve); age, \( \geq 60 \) or \( < 60 \) years; gender, male or female; serum \( \alpha \)-FP, \( \geq 400 \) or \( < 400 \); HBV+, with or without; HCV+, with or without; cirrhosis, with or without; tumour capsulation, with or without; tumour size, \( \geq 5 \) or \( < 5 \) cm; tumour number, solitary or \( \geq 2 \); grades, I+II or III+IV, pathologic stages, I+II or III+IV.

*\( P < 0.005 \).

\( \alpha \)-FP, \( \alpha \)-fetoprotein; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PCNA, proliferating cell nuclear antigen.
regulation of apoptosis. Mutations of p53 were described in malignant tumours and can be the cause of the alterations of this balance. A correlation between p53 and PCNA expression in HCC has been reported (33, 34), in our study as well. In general, p53 (1) correlates with increased PCNA LI in HCC and represents advanced disease states and poor outcome. Our result is consistent with previous reports (33, 34). It might be explained that the CDK inhibitor p21/WAF1 was known to be regulated by p53-dependent pathways. P21/WAF1's function is to bind with PCNA and inhibit the action of PCNA (28). Overexpression of p53 on immunostaining (mostly p53 mutation) might disturb this pathway and trigger PCNA activity, thereby promoting cancer cells' proliferation.

Kobayashi et al. (19) reported that p21/WAF1 mRNA expression levels of non-tumour tissues were significantly higher in HCV-positive cases than in HBV-positive cases, indicating that p21/WAF1 protein expression was affected by hepatitis virus type. In addition, reduced p21/WAF1 expression is frequently observed in HCC, either at the mRNA (18, 19) or protein level (20, 35). In HCC with a mutant p53 gene, the p21/WAF1 mRNA expression levels were significantly lower than those of the corresponding non-cancerous liver tissues, whereas the p21/WAF1 mRNA expression levels of HCCs with the wild-type p53 gene were not significantly different from the levels in the corresponding non-cancerous tissues (18, 26), suggesting that p21 expression may be regulated predominantly by p53 in HCCs. Compared with previous studies, our study demonstrates similar results on p53 and PCNA, but different p21/WAF1 expression in HCC. We found that overexpression of p21/WAF1 exists in HCC rather than surrounding non-tumourous tissues. P21/WAF1 is almost absent in the non-tumourous tissues. This is unique to major previous reports.

Many reports had focused on this issue and developed the possible events for p21/WAF1 expression activated by a p53-independent pathway (16, 36). For example, in human cancers, expression of p21/WAF1 has been reported to be independent of p53 and increased in pancreatic carcinoma (37), non-small cell lung carcinoma (38), and cutaneous squamous cell carcinoma (39). The possible explanation for increased p21/WAF1 may be induced through p53-independent manner, by serum, platelet-derived growth factor, fibroblast growth factor, epithelial growth factor (16), transforming growth factor-β (40), irradiation (41), and oxidative stress (41, 42). Further, mutation does not result in overexpression of p21/WAF1 in HCC and other cancers (43–45). Increased p21/WAF1 expression is probably because of

### Table 4. Correlation of clinicopathologic factors and disease-specific survival of HCC

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Univariate</th>
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<th>Multivariate</th>
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<td></td>
<td>Risk</td>
<td>95% CI</td>
<td>P</td>
<td>Risk</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>P21</td>
<td>1.82</td>
<td>1.07–3.11</td>
<td>0.024*</td>
<td>3.15</td>
<td>1.79–5.56</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>p53</td>
<td>2.23</td>
<td>1.34–3.71</td>
<td>0.008*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCNA</td>
<td>4.37</td>
<td>2.37–4.37</td>
<td>&lt; 0.001*</td>
<td>4.03</td>
<td>2.05–7.92</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Clinical parameters

| Age | 0.88 | 0.52–1.49 | NS | – | – | – |
| Gender | 0.67 | 0.63–1.65 | NS | – | – | – |
| AFP | 2.13 | 1.26–3.59 | 0.005* | – | – | – |
| HBV | 1.61 | 0.88–2.94 | NS | – | – | – |
| HCV | 0.64 | 0.35–1.16 | NS | – | – | – |
| Cirrhosis | 1.18 | 0.69–2.0 | NS | – | – | – |

Patho parameters

| Tumour capsule | 2.12 | 1.32–3.62 | 0.001* | – | – | – |
| Tumour size | 1.38 | 0.83–2.31 | NS | – | – | – |
| Tumour number | 1.28 | 0.71–2.29 | NS | – | – | – |
| Pathologic stages | 4.08 | 2.29–7.26 | < 0.001* | 3.37 | 1.86–6.10 | < 0.001* |
| Grade | 1.71 | 1.19–2.44 | 0.004* | – | – | – |

P21, positive or negative; p53, positive or negative; PCNA, high or low (by ROC curve); age, ≥60 or < 60 years; gender, male or female; serum αfP, ≥400 or < 400; HBV+, with or without; HCV+, with or without; cirrhosis, with or without; tumour capsule, with or without; tumour size, ≥5 or < 5 cm; tumour number, solitary or ≥2; grades, I+II or III+IV; pathologic stages, I+II or III+IV.

*P < 0.05.

AFP, α-fetoprotein; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PCNA, proliferating cell nuclear antigen.
enhanced posttranscriptional or posttranslational protein stabilization. Taken together, increased expression of p21/WAF1 in different types of cancers is regulated through a p53-independent pathway in contrast to reduced p21/WAF1 expression through a p53-dependent pathway. These might explain why the overexpression of p21/WAF1 did not relate to p53 overexpression in this study.

There is a discrepancy as to why there were no differences in the disease recurrence regarding p21/WAF1 expression, but overexpression of P21/WAF1 significantly related to the longer survival. Malignant transformation of hepatocyte is considered to be a multifactorial and multistep process involving different genetic alterations (1). Recurrence of HCC did need some genetic change that may be not relevant to p21/WAF1 because mutation is not a key factor in HCC and other cancers (43–45). The p21/WAF1 can interact with PCNA and directly inhibit PCNA-dependent DNA replication in the absence of a cyclin–CDK complex by blocking the ability of PCNA to activate DNA polymerase-δ, the principal replicative DNA polymerase (46). The possible explanation of overexpression of p21/WAF1 probably is in order to control the abnormal cell-cycle progression and to suppress replication of tumour cells, which has been demonstrated in that ectopic p21/WAF1 introduction suppressed the growth of HCC cell line from an in vitro study (47). It implies when the proliferating tumour cells contain increased PCNA, more p21/WAF1 may be required to bind PCNA and then to inhibit the activity of PCNA (48). Under high proliferating states of tumour (when tumour recurred), increased p21/WAF1 is just a reactive event to inhibit cell growth, and thereby prolong patients’ survival, as found in our study.

We wonder about the reason for diverse results of p21/WAF1 study on HCCs. It might be caused by different methodology, antibodies, patient groups, study areas, or pathogenic aetiologies. In our series, viral hepatitis B or C is the prime cause to induce cirrhosis and HCC. However, we did not find a difference in p21/WAF1 expression between hepatitis B and C patients. Nevertheless, we thought the results are convincing owing to the relatively large sample size and long-term follow-up of patients. Parallel and comparative study of mRNA and protein levels of p21/WAF1 might provide a further insight to answer these questions.

In conclusion, the present study shows that there is no correlation between p21/WAF1 and p53 expression in HCC. Increased p21/WAF1 expression in HCC was possibly a reactive effect to increased PCNA levels (tumour proliferation) in the tumour. Finally, overexpression of p21/WAF1 serves as an independent and good survival factor for HCC, which is a novel finding different from previous reports.

References