# Promoter mutations and cellular distribution of telomerase in non-clear cell and clear cell hepatocellular carcinoma

## Supplementary Materials

**Supplementary Table 1: Clinical characteristics of the enrolled patients with primary HCC (*n* = 316)**

| Variables | Number of patients (%) |
|---|---|
| Age (years) | |
| < 55 | 180 (57) |
| ≥ 55 | 136 (43) |
| Gender | |
| Male | 268 (85) |
| Female | 48 (15) |
| HBsAg | |
| Positive | 270 (85) |
| Negative | 46 (15) |
| Liver cirrhosis | |
| Yes | 231 (73) |
| No | 85 (27) |
| BCLC stage | |
| 0 and A | 102 (32) |
| B | 154 (49) |
| C | 60 (19) |
| Tumor size (cm) | |
| < 5 | 135 (43) |
| ≥ 5 | 181 (57) |
| Portal vein thrombosis | |
| Yes | 45 (14) |
| No | 271 (86) |
| Number of tumors | |
| Single | 255 (81) |
| Multiple | 61 (19) |
| α-Fetoprotein (μg/L) | |
| < 200 | 164 (52) |
| ≥ 200 | 152 (48) |
| Family history | |
| Yes | 36 (11) |
| No | 280 (89) |

*Abbreviations*: BCLC, Barcelona Clinic Liver Cancer.

## Supplementary Table 2: Characteristics of patients with HCC ($n$ = 316) according to the status of TERT promoter mutations

| Variables | TERT wild-type ($n$ = 213) | TERT mutant ($n$ = 103) | $P$ |
|---|---|---|---|
| Gender | | | |
| Male ($n$ = 268) | 174 (65%) | 94 (35%) | 0.026* |
| Female ($n$ = 48) | 39 (81%) | 9 (19%) | |
| Age (years), mean ± SD | 50.9 ± 11.6 | 54.2 ± 10.1 | 0.012* |
| HBsAg | | | |
| Negative ($n$ = 46) | 29 (63%) | 17 (37%) | 0.495 |
| Positive ($n$ = 270) | 184 (68%) | 86 (32%) | |
| HBV-DNA (IU/ml) | | | |
| ≤ 2000 ($n$ = 218) | 140 (64%) | 78 (36%) | 0.432 |
| > 2000 ($n$ = 42) | 30 (71%) | 12 (29%) | |
| Cirrhosis | | | |
| Yes ($n$ = 224) | 156 (70%) | 68 (30%) | 0.157 |
| No ($n$ = 85) | 52 (61%) | 33 (39%) | |
| α-Fetoprotein (μg/L) | | | |
| ≤ 200 ($n$ = 164) | 104 (63%) | 60 (37%) | 0.116 |
| > 200 ($n$ = 152) | 109 (72%) | 43 (28%) | |
| Tumor differentiation | | | |
| Well ($n$ = 23) | 20 (87%) | 3 (13%) | 0.028* |
| Moderate ($n$ = 137) | 97 (71%) | 40 (29%) | |
| Poor ($n$ = 156) | 96 (62%) | 60 (38%) | |
| BCLC stage | | | |
| 0 and A ($n$ = 102) | 76 (75%) | 26 (25%) | 0.038* |
| B ($n$ = 154) | 104 (68%) | 50 (32%) | |
| C ($n$ = 60) | 33 (55%) | 27 (45%) | |
| Tumor size (cm) | | | |
| < 5 ($n$ = 135) | 92 (68%) | 43 (32%) | 0.808 |
| ≥ 5 ($n$ = 181) | 121 (67%) | 60 (33%) | |
| Portal vein thrombosis | | | |
| Yes ($n$ = 45) | 26 (58%) | 19 (42%) | 0.137 |
| No ($n$ = 271) | 187 (69%) | 84 (31%) | |
| Number of tumors | | | |
| Single ($n$ = 255) | 173 (68%) | 82 (32%) | 0.734 |
| Multiple ($n$ = 61) | 40 (66%) | 21 (34%) | |
| Family history | | | |
| Yes ($n$ = 36) | 23 (64%) | 13 (36%) | 0.633 |
| No ($n$ = 280) | 190 (68%) | 90 (32%) | |
| TERT nuclear expression | | | |
| Positive ($n$ = 113) | 67 (59%) | 46 (41%) | 0.405 |
| Negative ($n$ = 64) | 42 (66%) | 22 (34%) | |
| TERT cytoplasmic expression | | | |
| Positive ($n$ = 169) | 105 (62%) | 64 (38%) | 0.486# |
| Negative ($n$ = 8) | 4 (50%) | 4 (50%) | |

Data were expressed as number (%) and were analyzed using the $\chi 2$-test except that the $P$ value of Age (years) was calculated using an unpaired student's $t$ test. One hundred and seventy seven cases were used for TERT nuclear and cytoplasmic localization analysis. * indicates statistical significant ($P < 0.05$). # indicates the $P$ value was calculated from Fisher's exact test. *Abbreviations*: BCLC, Barcelona Clinic Liver Cancer.

**Supplementary Table 3: Multivariate analysis of clinical pathological factors associated with TERT promoter mutations in patients with HCC (*n* = 316)**

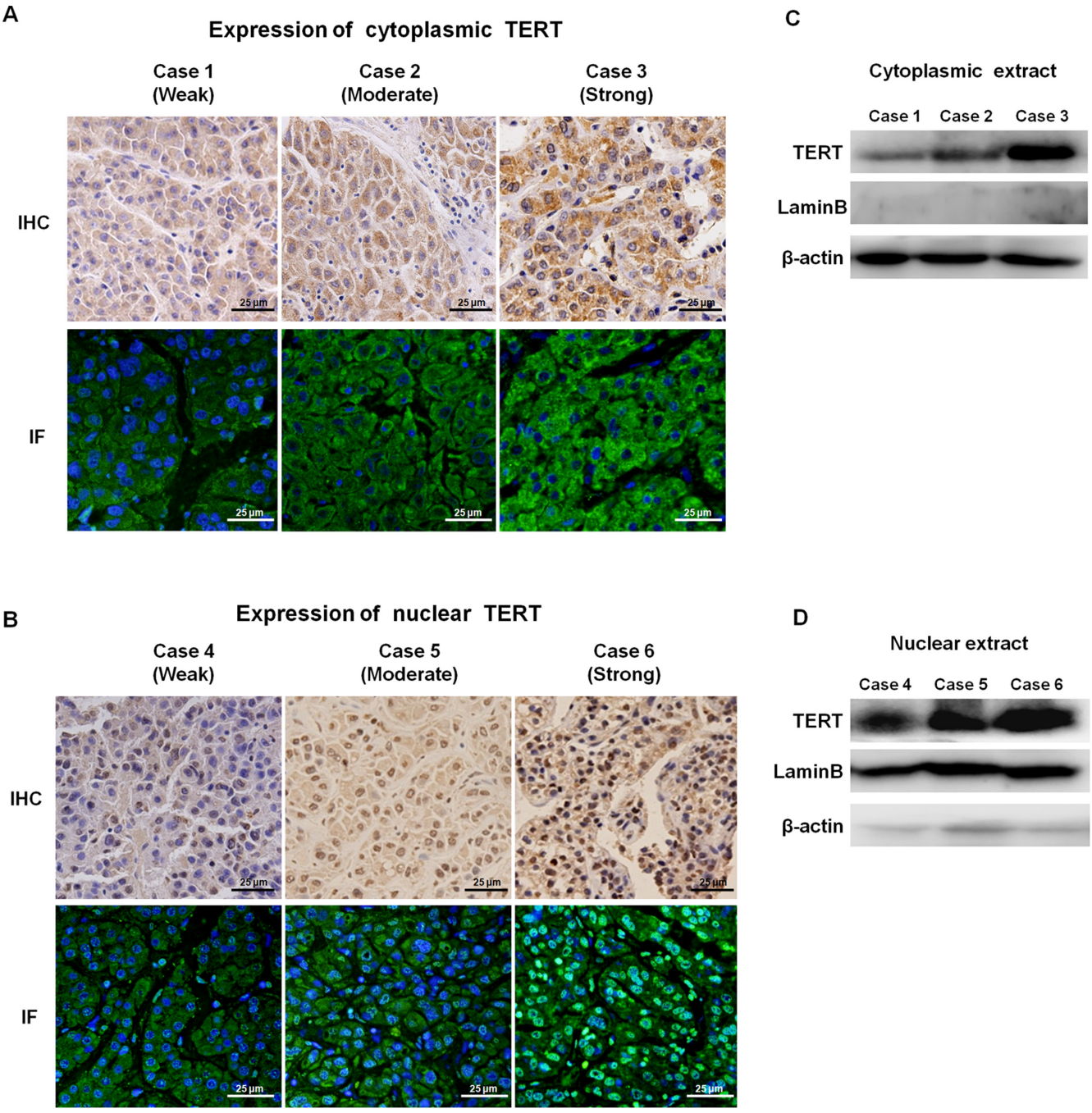
| Variables | Odds ratio | 95.0% CI | P |
|---|---|---|---|
| Age (years) | | | |
| < 55 | 1.00 | | |
| ≥ 55 | 2.24 | 1.26 to 4.00 | 0.006* |
| Gender | | | |
| Female | 1.00 | | |
| Male | 2.00 | 0.85 to 4.71 | 0.112 |
| α-Fetoprotein (μg/L) | | | |
| ≤ 200 | 1.00 | | |
| > 200 | 0.69 | 0.38 to 1.25 | 0.222 |
| Tumor differentiation | | | |
| Well and Moderate | 1.00 | | |
| Poor | 1.71 | 0.96 to 3.04 | 0.067 |
| BCLC stage | | | |
| 0 and A | 1.00 | | |
| B | 1.84 | 0.72 to 4.70 | 0.203 |
| C | 6.08 | 1.17 to 31.49 | 0.032* |
| Cirrhosis | | | |
| No | 1.00 | | |
| Yes | 0.48 | 0.25 to 0.92 | 0.027* |
| HBsAg | | | |
| Negative | 1.00 | | |
| Positive | 0.92 | 0.35 to 2.42 | 0.862 |
| HBV-DNA (IU/ml) | | | |
| ≤ 2000 | 1.00 | | |
| > 2000 | 0.73 | 0.33 to 1.60 | 0.424 |
| Portal vein thrombosis | | | |
| No | 1.00 | | |
| Yes | 0.70 | 0.16 to 3.07 | 0.639 |
| Tumor size (cm) | | | |
| < 5 | 1.00 | | |
| ≥ 5 | 0.57 | 0.25 to 1.33 | 0.196 |
| Number of tumors | | | |
| Single | 1.00 | | |
| Multiple | 0.94 | 0.44 to 2.04 | 0.883 |
| Family history | | | |
| No | 1.00 | | |
| Yes | 1.07 | 0.45 to 2.58 | 0.873 |

Odds ratios and 95% CIs were calculated using multivariate logistic regression * indicates statistical significant ($P < 0.05$).
*Abbreviations*: CI, confidence interval.

**Supplementary Table 4: Multivariate cox regression analysis for risk factors associated with disease-free survival of HCCs (*n* = 275)**

| Variables | Hazard ratio | 95.0% CI | *P* |
|---|---|---|---|
| TERT promoter mutations | | | |
| Wild-type | 1.00 | | |
| Mutant | 1.72 | 1.23 to 2.39 | 0.001* |
| α-Fetoprotein (μg/L) | | | |
| ≤ 200 | 1.00 | | |
| > 200 | 2.06 | 1.46 to 2.92 | < 0.001* |
| Tumor differentiation | | | |
| Well and Moderate | 1.00 | | |
| Poor | 0.93 | 0.66 to 1.31 | 0.673 |
| BCLC stage | | | |
| 0 and A | 1.00 | | |
| B and C | 1.57 | 0.88 to 2.79 | 0.126 |
| Portal vein thrombosis | | | |
| No | 1.00 | | |
| Yes | 2.18 | 1.40 to 3.39 | 0.001* |
| Tumor size (cm) | | | |
| < 5 | 1.00 | | |
| ≥ 5 | 1.25 | 0.76 to 2.05 | 0.379 |
| Number of tumors | | | |
| Single | 1.00 | 1.14 to 2.47 | |
| Multiple | 1.68 | | 0.008* |

Hazard ratios and 95% CIs were calculated using multivariate Cox regression model.
*indicates statistical significant ($P < 0.05$). *Abbreviations*: CI, confidence interval.




**Supplementary Figure 1: TERT nuclear and cycoplasmic expression evaluated by immunohistochemistry, immunofluorescence and western blot.** Representative images of IHC (immunohistochemical staining, upper panel) and IF (immunofluorescence staining, lower panel) of three cases of HCC demonstrated the expression level of TERT (weak, moderate and strong) in cytoplasm **(A)** and in nucleus **(B)**. TERT was probed with green fluorescence and the nucleus was stained with DAPI (blue). The cytoplasmic extract protein samples from case 1–3 and nuclear extract protein samples from case 4–6 were analyzed by western blot to confirm the expression level of cytoplasmic TERT **(C)** and nuclear TERT **(D)**. β-actin was used as cytoplasmic loading control for cytoplasmic extracts and laminB was used as nuclear loading control for nuclear extracts.