Diabetes, an independent poor prognostic factor of non-B non-C hepatocellular carcinoma, correlates with dihydropyrimidinase-like 3 promoter methylation

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Supplemental Table S1. Univariate analysis of diabetes therapeutic factors and disease-specific survival after resection of NBNC hepatocellular carcinoma in diabetic patients (log-rank test).

Variable	Median DSS (month)	*P* value
Diet therapy: (-) vs (+)	42.0 vs 45.5	0.676
Oral hypoglycemic agents: (-) vs (+)	44.0 vs 43.0	0.965
Metoformin: (-) vs (+)	41.0 vs 52.5	0.297
Insulin: (-) vs (+)	45.5 vs 37.0	0.308

Supplemental Table S2. Univariate analysis of clinicopathological factors and overall survival after resection of NBNC hepatocellular carcinoma (log-rank test).

Variable	Median OS (month)	*P* value
Age: ＜68 vs ≧68	56.5 vs 47.0	0.514
Male vs Female	56.0 vs 47.5	0.582
BMI: ＜23.9 vs ≧23.9	48.0 vs 52.0	0.479
Tumor multiplicity: single vs multiple	52.0 vs 45.5	0.015
Tumor size (mm):<20 vs ≧20	59.0 vs 47.5	0.304
Tumor differentiation: wel, mod vs por	47.5 vs 53.0	0.105
Growth type:　Expansive growth vs invasive growth	51.5 vs 46.0	0.976
Serosal infiltration: (-) vs (+)	53.0 vs 38.0	0.023
Formation of capsule: (-) vs (+)	69.5 vs 45.5	0.022
Infiltration to capsule: (-) vs (+)	59.0 vs 45.5	0.390
Septum formation: (-) vs (+)	59.0 vs 46.5	0.112
Bile duct invasion: (-) vs (+)	51.0 vs 52.0	0.787
Portal vein invasion: (-) vs (+)	54.0 vs 44.0	0.005
Hepatic vein invasion: (-) vs (+)	56.5 vs 43.0	0.001
T1-2 vs T3-4 (UICC)	51.5 vs 41.0	0.404
Alcoholic liver damage: (-) vs (+)	51.5 vs 52.5	0.832
Alcohol habit: (-) vs (+)	46.0 vs 54.0	0.228
History of T2DM: (-) vs (+)	62.0 vs 43.0	0.007
HbA1c (%): <6.1 vs ≧6.1	50.5 vs 47.0	0.745
Blood glucose (mmol/L): <112.5 vs ≧112.5	60.0 vs 43.0	0.074
History of hypertension: (-) vs (+)	98.9 vs 73.0	0.092
History of dyslipidemia: (-) vs (+)	98.0 vs 47.0	0.083
Smoking habits (overall): (-) vs (+)	104.0 vs 98.0	0.947

Supplemental Table S2. continued

Smoking habits (Brinkman index≧400): (-) vs (+)	104.0 vs 98.0	0.901
DPYSL3 promoter methylation: (-) vs (+)	62.0 vs 45.5	0.009
CDK2NA promoter methylation: (-) vs (+)	54.0 vs 43.0	0.552

OS; Overall survival, BMI; body mass index, T2DM; type2 diabetes, wel; well-differentiated adenocarcinoma, mod; moderate-differentiated adenocarcinoma, por; poorly-differentiated adenocarcinoma.

Supplemental Table S3. Univariate analysis of diabetes therapeutic factors and overall survival after resection of NBNC hepatocellular carcinoma in diabetic patients (log-rank test).

| Variable	Median DSS (month)	*P* value
Diet therapy: (-) vs (+)	64.0 vs 51.0	0.98
Oral hypoglycemic agents: (-) vs (+)	47.0 vs 75.0	0.474
Metoformin: (-) vs (+)	61.0 vs 76.0	0.206
Insulin: (-) vs (+)	61.0 vs 36.0	0.788

Supplemental Table S4. Multivariate analysis of clinicopathological factors and overall survival after resection of NBNC hepatocellular carcinoma (Cox proportional hazards model)

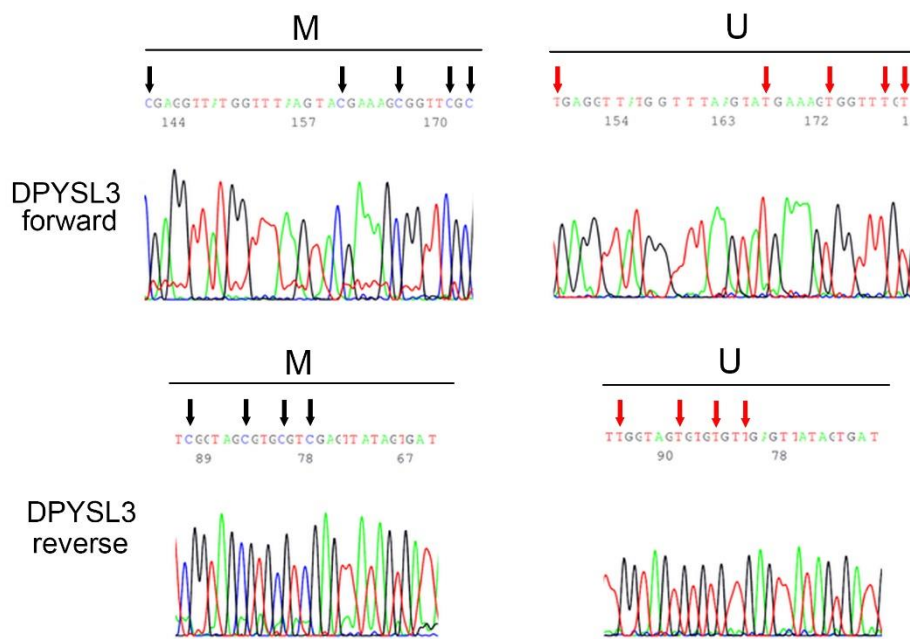| Variable	Hazard ratio	95%CI	*P* value
Hepatic vein invasion	2.872	1.301－6.338	0.009
Serosal infiltration	2.482	1.076－5.723	0.033
History of T2DM	2.221	1.032－5.210	0.045

95% CI; confidence interval, T2DM; type2 diabetes.
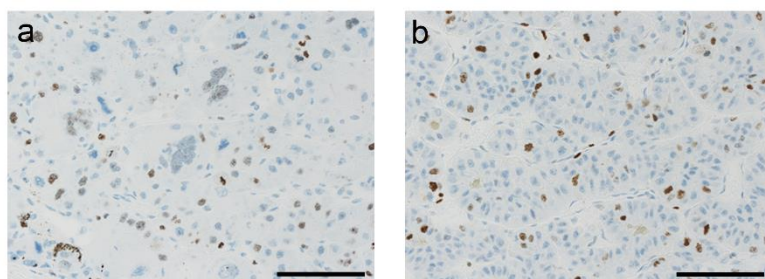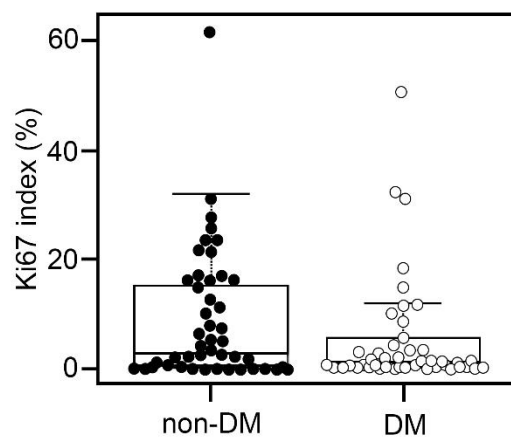
Supplemantal Figure S1.

a
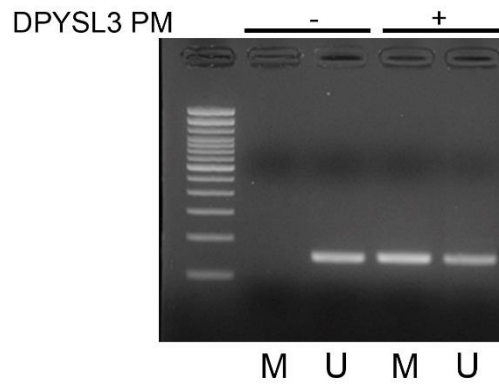
b

Supplemental Figure S2.

Legends for supplemental figures

Supplemental Figure S1.

Analysis by methylation-specific PCR in NBNC-HCC.

The subjects bearing promoter methylation (PM) of DPYSL3 showed methylated and unmethylated bands (**a**). Non-methylated tumor exhibited only unmethylated band. U = unmethylated, M = methylated. Sequencing of the PCR amplicons indicated methylated base of cytosine as C (black arrows), whereas C in unmethylated DNA was converted to thymidine (T) after bisulfide treatment (red arrows) (**b**). Lines under nucleotide sequence indicate primer sequence.

Supplemental Figure S2.

Cell proliferation analysis evaluated by Ki67 immunostaining

Ki67 was positive in the nucleus of hepatocellular carcinoma cells in both non-DM cases (**a**) and DM cases (**b**). Ki67 index was comparable between non-DM and DM (**c**). Bar represents 50 μm in each.