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ORIGINAL ARTICLE

# HOXD10 expression as a prognostic factor for hepatocellular carcinoma treated with curative resection

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## Summary

***Purpose:*** *HOXD10 downregulation resulting from epigenetic changesas well as its role as a tumor suppressor have been reported in several cancers including hepatocellular carcinomas (HCCs). However, the prognostic role of HOXD10 expression in HCC tissue samples has not been evaluated.*

***Methods:*** *HOXD10 expression was investigated in 278 curatively resected HCC samples using immunohistochemistry and its effectiveness in predicting patient outcome was analyzed.*

***Results:*** *Low expression of HOXD10 was observed in 82.7% of HCC samples, and this was associated with increased age, large tumor size and advanced stage.HOXD10 was an independent predictive factor for early tumor recurrence at less than 2 years. Patients with low HOXD10 expression showed shorter recurrence-free survival (RFS) (p=0.024) and disease-specific survival (DSS) (p=0.016) than those with high expression. Multivariate analysis confirmed that low HOXD10 expression was an independent predictor of shorter RFS (hazard ratio 1.873, p=0.006) and DSS (hazard ratio2.504, p=0.012) than high HOXD10 expression.*

***Conclusions:*** *The present study provides clinical evidence supporting the use of HOXD10 as a prognostic biomarker in curatively resected HCCs, and suggests that HOXD10 could also be a potential therapeutic target in HCC.*

***Key words:*** *hepatocellular carcinoma, prognosis, recurrence, HOXD10, biomarkers*

## Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth most common cause of cancer-related deaths worldwide [1]. Although surgical resection is the treatment of choice for HCC, prognosis after hepatectomy is poor due to the high frequency of tumor recurrence [2]. Sorafenib has been used as the most effective systemic treatment for advanced HCC s[3,4], and novel targeted agents, such as regorafenib and lenvatinib, or immune checkpoint inhibitors, such as nivolumab and pembrolizumab, have been approved by the US Food and Drug Administration [5-8]. However, the application of these therapies remains limited [4,6], and reliable molecular biomarkers and therapeutic target agents need to be identified in the era of precision medicine [9].

Homeobox D10 (*HOXD10*) is a member of the homeobox gene family, which encodes homeoproteins that serve as transcription factors and play an important role in carcinogenesis by regulating cell growth, cell cycle progression, and apoptosis [10]. *HOXD10* is known to be downregulated by epigenetic mechanisms and play an important role as a tumor suppressor protein in some types of cancer including cholangiocellular carcinoma, prostate cancer, thyroid papillary cancer, colon can-

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cer, pancreatic cancer and endometrial cancer [11-16]. A recent study by Guo et al revealed frequent downregulation of *HOXD10* by hypermethylation in HCC and its association with shorter patient survival [17]. However, the prognostic role of *HOXD10* protein expression in HCC tissue samples has not been reported.

In this study, we aimed to evaluate *HOXD10* protein expression in surgically resected HCC samples using immunohistochemistry (IHC) and analyze its effectiveness in predicting patient outcomes.

## Methods

*Patients and specimens*

A total of 283 patients treated with curative hepatectomy as a first line of treatment for primary HCC between July 2000 and May 2006 at the Samsung Medical Center, Seoul, Korea were enrolled in this study. Five patients who presented with insufficient tissue on the tissue microarray (TMA) were excluded, leaving 278 patients within the study cohort.

Curatively resected tumors had complete resection margins (confirmed by microscopic examination) and no residual tumors (confirmed by radiological examination one month after surgery). All tumor tissues were confirmed by histology and tumor staging was performed according to both the American Joint Committee on Cancer (AJCC) staging system (8$^{th}$ edition) [16] and Barcelona Clinic Liver Cancer (BCLC) staging classification [19]. Intrahepatic metastasis and multicentric occurrence were defined according to previously reported criteria [20], and tumor necrosis or tumor-infiltrating lymphocytes (TILs) were identified by hematoxylin and eosin staining as described previously [21]. All patients were followed up every 3 months after surgery, with three-phase dynamic computed tomography scans or magnetic resonance imaging as well as serum α-fetoprotein (AFP) level evaluations. Recurrence-free survival (RFS) or disease-specific survival (DSS) was defined as the difference between the date of surgery and date of recurrence or HCC-related death, respectively [20]. The Institutional Review Board of the Samsung Medical Center approved this study and waived the need for informed consent (IRB No. 2020-09-161).

*IHC*

IHC was performed using TMA consisting of two 2 mm cores of HCC tissue as previously described [23]. The sections were incubated with a rabbit anti-HOXD10 antibody (aa201-230, 1:150, LifeSpan BioSicenes, Seattle WA, USA) for 15 min in a Bond-max autoimmunostainer (Leica Biosystems, Melbourne, Australia) after antigen retrieval with 100 ml of ER1 buffer (Leica Biosystems). Antigen-antibody chromogenic reactions were developed for 10 min using the Bond™ Polymer refine detection kit, DS9800 (Vision Biosystems, Melbourne, Australia). Normal prostate tissues were used as a control. Nuclear staining with moderate to strong intensity in more than 5% of the tumor cells was defined as high expression (Figure 1).

*Statistics*

Pearson's chi square test, Fisher's exact test or Cochran-Armitage test were used to analyze the relationship between *HOXD10* expression and clinicopathologic parameters, as appropriate. Logistic regression analysis was used to predict early recurrence, and the Kaplan-Meier survival method was used to analyze survival rates with any differences compared using the log-rank test. Cox's proportional hazard regression model was used to assess the association between clinicopathologic factors and survival time. Prognostic factors with p values less than 0.05 in the univariate analysis were carried over into the multivariate analysis. We examined the proportional hazard assumption graphically to determine whether variables in the Cox proportional hazard model were constants independent of time. Two-sided p values less than 0.05 were considered statistically significant. Statistical analyses were conducted using IBM SPSS v25.0 for Windows (IBM Corp., Armonk, NY, USA) and R software.

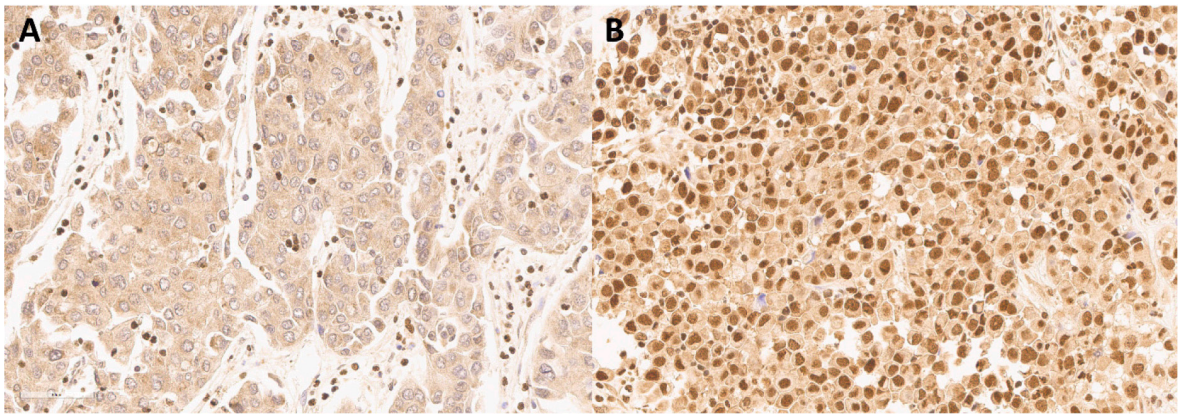


**Figure 1.** Representative figures of HOXD10 immunohistochemistry. **A:** Low expression samples: Nuclei of tumor cells were negative for HOXD10. **B:** High expression samples: Tumor cells show nuclear staining for HOXD10.

## Results

*HOXD10 expression in HCC tissues and its association with clinicopathologic features*

*HOXD10* expression was defined as high in 48 cases (17.3%) and low in 230cases (82.7%) within our study population. The clinicopathologic features of the study population and association of these features with *HOXD10* expression are summarized in Table 1.

A total of 230 (82.7%) male and 48 (17.3%) female patients were enrolled in this study, and their age at diagnosis ranged from 17 to 76 (median: 53) years. The largest tumor size was greater than 5 cm in 94 (33.8%) cases. On histopathological examination, the tumors in 222 (79.9%) cases demonstrated Edmondson grade II differentiation. Microvascular or major portal vein invasion was observed in 152 (54.7%) and 11 (4%) cases, respectively, while 64(23.0%) patients presented with intrahepatic metastasis and another 17(6.1%) had multicentric tumors. Necrosis was detected in 67 (24.1%) cases and TIL was detected in 86 (31.1%) cases. The tumors in our cohort were then staged using the AJCC 8th edition parameters as follows: T1a, 40 (14.4%) cases; T1b, 85 (30.6%); T2, 109 (39.2%); T3, 33 (11.9%); and T4, 11(4.0%) cases. The laboratory data showed that 29 (10.4%) patients were hypoalbuminemic and 99 (36.9%) patients had elevated AFP levels (>200 ng/mL). Most of the patients had underlying hepatitis B or C virus infection, with only 37 (13.3%) patients testing negative for both viruses;140 (50.4%) patients had a history of cirrhotic liver disease. Approximately half of the study population (138 patients, 49.6%) experienced tumor recurrence before 2 years after surgery and 49 (35%) of the remaining patients experienced recurrence after 2 years of surgery.

Low *HOXD10* expression was significantly associated with increased age (>55 years, p=0.008), large tumor size (>5.0 cm; p<0.001), advanced AJCCT (p=0.007) and BCLC stages (p<0.001), a history of liver cirrhosis (p=0.013), and early recurrence (p=0.013).

*HOXD10 expression is a predictor of early recurrence*

Using univariate logistic regression analysis, we were able to determine that increased tumor size (>5.0cm; p<0.001), high Edmondson grade (III; p=0.005), high serum AFP levels (>200ng/mL; p=0.032), and the presence of viral infection (p=0.011) can predict early tumor recurrence in clinical samples. Histopathologic features, such as the presence of microvascular invasion (p<0.001), major portal vein invasion (p=0.024), intrahepatic metastasis (p<0.001), necrosis (p<0.001), low TIL

**Table 1.** The association between HOXD10 expression and clinicopathologic parameters

| *Parameters* | *HOXD10 expression* | | | |
|---|---|---|---|---|
| | *Total, n=278* *n(%)* | *Low, n=230 (82.7%)* *n(%)* | *High, n=48 (17.3%)* *n(%)* | *p value* |
| Age, year | | | | |
| ≤55 | 161 (57.9) | 125 (77.6) | 36 (22.4) | 0.008 |
| >55 | 117 (42.1) | 105 (89.7) | 12 (10.3) | |
| Gender | | | | |
| Female | 48 (17.3) | 39 (81.3) | 9 (18.8) | 0.765 |
| Male | 230 (82.7) | 191 (83.0) | 39 (17.0) | |
| Tumor size, cm | | | | |
| ≤5.0 | 184 (66.2) | 141 (76.6) | 43 (23.4) | < 0.001 |
| >5.0 | 94 (33.8) | 89 (94.7) | 5 (5.3) | |
| Edmondson grade | | | | |
| I | 32 (11.5) | 27 (84.4) | 5 (15.6) | 0.111 |
| II | 222 (79.9) | 187 (84.2) | 35 (15.8) | |
| III | 24 (8.6) | 16 (66.7) | 8 (33.3) | |
| Microvascular invasion | | | | |
| (-) | 126 (45.3) | 100 (79.4) | 26 (20.6) | 0.176 |
| (+) | 152 (54.7) | 130 (85.5) | 22 (14.5) | |

*Continued on the next page*

| *Parameters* | *HOXD10 expression* | | | |
|---|---|---|---|---|
| | *Total, n=278 n(%)* | *Low, n=230 (82.7%) n(%)* | *High, n=48 (17.3%) n(%)* | *p value* |
| Major portal vein invasion[b] | | | | |
| (-) | 267 (96.0) | 219 (82.0) | 48 (18.0) | 0.221 |
| (+) | 11 (4.0) | 11 (100.0) | 0 (0.0) | |
| Intrahepatic metastasis | | | | |
| (-) | 214 (77.0) | 172 (80.4) | 42 (19.6) | 0.057 |
| (+) | 64 (23.0) | 58 (90.6) | 6 (9.4) | |
| Multicentric occurrence[b] | | | | |
| (-) | 261 (93.9) | 215 (82.4) | 46 (17.6) | 0.746 |
| (+) | 17 (6.1) | 15 (88.2) | 2 (11.8) | |
| Necrosis | | | | |
| (-) | 211 (75.9) | 174 (82.5) | 37 (17.5) | 0.833 |
| (+) | 67 (24.1) | 56 (83.6) | 11 (16.4) | |
| TIL | | | | |
| (-) | 192 (69.1) | 161 (83.9) | 31 (16.1) | 0.600 |
| (+) | 55 (19.8) | 45 (81.8) | 10 (18.2) | |
| (++) | 31 (11.1) | 24 (77.4) | 7 (22.6) | |
| AJCC T-stage[c] | | | | |
| 1a | 40 (14.4) | 27 (67.5) | 13 (32.5) | 0.007 |
| 1b | 85 (30.6) | 73 (85.9) | 12 (14.1) | |
| 2 | 109 (39.2) | 88 (80.7) | 21 (19.3) | |
| 3 | 33 (11.9) | 31 (93.9) | 2 (6.1) | |
| 4 | 11 (4.0) | 11 (100.0) | 0 (0.0) | |
| BCLC stage[c] | | | | |
| 0-A | 159 (57.2) | 119 (74.8) | 40 (25.2) | <0.001 |
| B | 106 (38.1) | 98 (92.5) | 8 (7.5) | |
| C | 13 (4.7) | 13 (100.0) | 0 (0.0) | |
| Albumin level, g/dL | | | | |
| >3.5 | 249 (89.6) | 207 (83.1) | 42 (16.9) | 0.606 |
| ≤3.5 | 29 (10.4) | 23 (79.3) | 6 (20.7) | |
| AFP level, ng/mL | | | | |
| ≤200 | 169 (63.1) | 141 (83.4) | 28 (16.6) | 0.586 |
| >200 | 99 (36.9) | 80 (80.8) | 19 (19.2) | |
| Etiology[b] | | | | |
| Non-viral | 37 (13.3) | 31 (83.8) | 6 (16.2) | 1.000 |
| HBV | 211 (75.9) | 173 (82.0) | 38 (18.0) | |
| HCV | 26 (9.4) | 22 (84.6) | 4 (15.4) | |
| HBV & HCV | 4 (1.4) | 4 (100.0) | 0 (0.0) | |
| Liver cirrhosis | | | | |
| (-) | 138 (49.6) | 122 (88.4) | 16 (11.6) | 0.013 |
| (+) | 140 (50.4) | 108 (77.1) | 32 (22.9) | |
| Early recurrence (≤2 years) | | | | |
| (-)[a] | 140 (50.4) | 108 (77.1) | 32 (22.9) | 0.013 |
| (+) | 138 (49.6) | 122 (88.4) | 16 (11.6) | |
| Late recurrence (>2 years) | | | | |
| (-)[a] | 91 (65.0) | 70 (76.9) | 21 (23.1) | 0.933 |
| (+) | 49 (35.0) | 38 (77.6) | 11 (22.4) | |

TIL: tumor infiltrating lymphocytes; AJCC: American Joint Committee on Cancer; BCLC: Barcelona Clinic Liver Cancer; AFP: α-fetoprotein; HBV: hepatitis B virus; HCV: hepatitis C virus.
[a]No early or late recurrence, [b]by Fisher's exact test, [c]by Cochran-Armitage trend test, otherwise by chi-square test

levels (p=0.003), and low *HOXD10* expression (p=0.015), were all significantly associated with early tumor recurrence. The results of multivariate analysis showed that intrahepatic metastasis, necrosis, and low *HOXD10* expression were independent predictive factors for early tumor recurrence (odds ratio 2.260 (95% confidence interval (CI): 1.037-4.925), p=0.040) (Table 2).

*Impact of HOXD10 expression on the survival of HCC patients*

Patients with low *HOXD10* expression presented with shorter RFS (p=0.024, Figure 2A) and DSS (p=0.016, Figure 2B) than those with high *HOXD10* expression. Consequently, we attempted to evaluate the predictive value of *HOXD10* expression for RFS and DSS. The results of univariate analysis

**Table 2.** Univariate and multivariate logistic regression models for predicting early tumor recurrence

| *Variables* | *Univariate model* | | | *Multivariate model* | | |
|---|---|---|---|---|---|---|
| | *Coefficient* | *OR (95% CI)* | *p value* | *Coefficient* | *OR (95% CI)* | *p value* |
| Age (>55 yr vs. ≤55 yr) | 1.056 | 0.656-1.700 | 0.823 | | | |
| Sex (male vs. female) | 1.087 | 0.583-2.026 | 0.793 | | | |
| Tumor size (>5.0 cm vs. ≤5.0 cm) | 3.171 | 1.877-5.357 | <0.001 | 1.361 | 0.691-2.680 | 0.372 |
| Edmondson grade (III vs. I+II) | 4.311 | 1.562-11.901 | 0.005 | 2.675 | 0.799-8.951 | 0.110 |
| Microvascular invasion (yes vs. no) | 3.902 | 2.364-6.440 | <0.001 | 0.991 | 0.499-1.966 | 0.979 |
| Major portal vein invasion (yes vs. no) | 10.859 | 1.371-86.024 | 0.024 | 0.716 | 0.071-7.246 | 0.777 |
| Intrahepatic metastasis (yes vs. no) | 16.192 | 6.683-39.231 | <0.001 | 12.713 | 5.146-31.404 | <0.001 |
| Multicentric occurrence (yes vs. no) | 0.533 | 0.191-1.484 | 0.228 | | | |
| Necrosis (yes vs. no) | 4.111 | 2.224-7.601 | <0.001 | 2.887 | 1.436-5.803 | 0.003 |
| TIL (no vs. yes) | 2.233 | 1.322-3.773 | 0.003 | 1.212 | 0.651-2.256 | 0.545 |
| Albumin level (≤3.5 g/dL vs. >3.5 g/dL) | 0.482 | 0.215-1.078 | 0.075 | | | |
| AFP level[a] (>200 ng/mL vs. ≤200 ng/mL) | 1.731 | 1.048-2.859 | 0.032 | 0.856 | 0.455-1.608 | 0.628 |
| Etiology (viral vs. non-viral) | 2.633 | 1.245-5.569 | 0.011 | 1.800 | 0.761-4.259 | 0.181 |
| Liver cirrhosis (yes vs. no) | 1.374 | 0.857-2.202 | 0.187 | | | |
| HOXD10 expression (low vs high) | 2.259 | 1.175-4.343 | 0.015 | 2.260 | 1.037-4.925 | 0.040 |

OR: odds ratio; CI: confidence interval; TIL: tumor infiltrating lymphocytes; AJCC: American Joint Committee on Cancer; BCLC: Barcelona Clinic Liver Cancer; AFP: α-fetoprotein
[a]Partial data were not available, and statistics were based on the available data

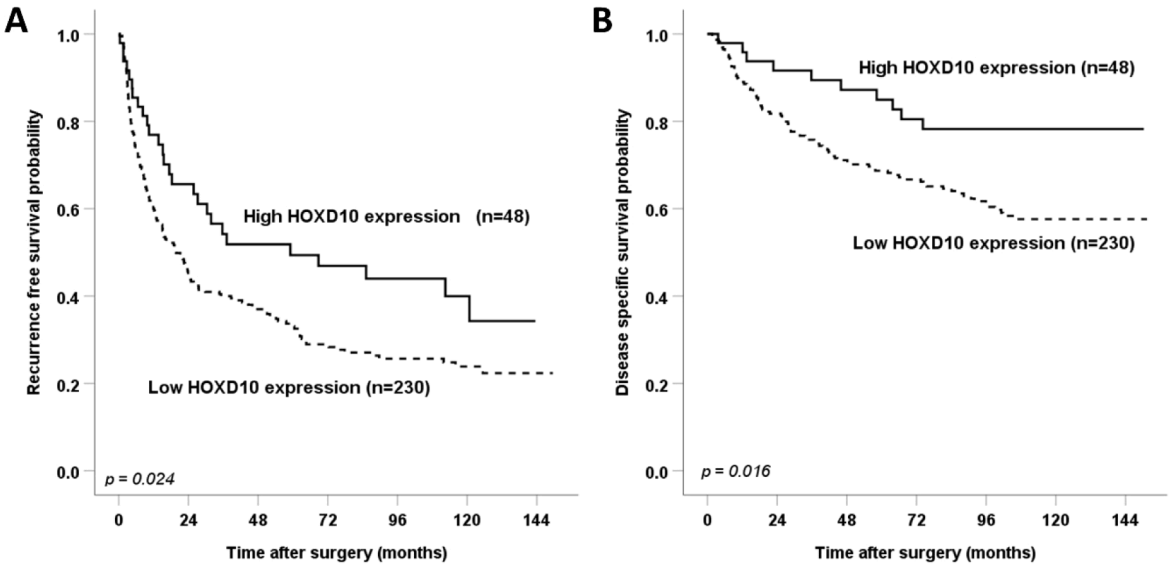


**Figure 2.** Kaplan-Meier survival curves according to HOXD10 expression. **A:** Recurrence free survival **B:** Disease-specific survival.

performed using Cox proportional hazard regression model revealed that (Table 3) large tumor size (>5.0cm; p<0.001), high Edmondson grade (III; p<0.001), microvascular invasion (p<0.001), major portal vein invasion (p<0.001), intrahepatic metastasis (p<0.001), necrosis (p<0.001), low TIL levels (p=0.024), advanced AJCC T (2,3,4 vs. 1; p=0.021 for RFS and p=0.001 for DSS) and BCLC stages (B,C vs. 0,A; p<0.001 for both RFS and DSS), hypoalbuminemia (p=0.008 for RFS and p=0.006 for DSS), high serum AFP levels (>200ng/mL; p=0.001 for RFS and p=0.007 for DSS), and low *HOXD10* expression (p=0.025 for RFS and p=0.019 for DSS) were associated with shorter RFS and DSS. Hepatitis virus infection (p=0.007) and a history of cirrhosis (p=0.060) also reduced RFS but did not affect DSS.

**Table 3.** Univariate analysis for recurrence free survival and disease specific survival

| *Variables* | *Recurrence free survival* | | *Disease specific survival* | |
|---|---|---|---|---|
| | *HR (95% CI)* | *p value* | *HR (95% CI)* | *p value* |
| Age (>55 yr vs. ≤55 yr) | 0.979 (0.730-1.312) | 0.885 | 0.932 (0.623-1.395) | 0.733 |
| Sex (male vs. female) | 1.057 (0.729-1.533) | 0.769 | 1.246 (0.729-2.130) | 0.421 |
| Tumor size (>5.0 cm vs. ≤5.0 cm) | 1.750 (1.303-2.350) | <0.001 | 2.896 (1.946-4.310) | <0.001 |
| Edmondson grade (III vs. I+II) | 2.385 (1.511-3.764) | <0.001 | 2.719 (1.542-4.795) | 0.001 |
| Microvascular invasion (yes vs. no) | 2.106 (1.566-2.832) | <0.001 | 3.146 (2.007-4.933) | <0.001 |
| Major portal vein invasion (yes vs. no) | 3.325 (1.751-6.312) | <0.001 | 4.910 (2.461-9.796) | <0.001 |
| Intrahepatic metastasis (yes vs. no) | 4.825 (3.481-6.689) | <0.001 | 5.675 (3.782-8.515) | <0.001 |
| Multicentric occurrence (yes vs. no) | 1.151 (0.608-2.180) | 0.666 | 0.653 (0.240-1.775) | 0.403 |
| Necrosis (yes vs. no) | 2.560 (1.860-3.525) | <0.001 | 4.394 (2.941-6.567) | <0.001 |
| TIL (no vs. yes) | 1.446 (1.050-1.992) | 0.024 | 2.426 (1.454-4.047) | 0.001 |
| AJCC T-stage (2,3,4 vs 1) | 1.643 (1.077-2.505) | 0.021 | 5.568 (2.046-15.153) | 0.001 |
| BCLC stage (B,C vs 0, A) | 2.123 (1.590-2.833) | <0.001 | 3.902 (2.562-5.943) | <0.001 |
| Albumin level (≤3.5 g/dL vs. >3.5 g/dL) | 0.552 (0.356-0.855) | 0.008 | 0.460 (0.265-0.799) | 0.006 |
| AFP level[a] (>200 ng/mL vs. ≤200 ng/mL) | 1.654 (1.231-2.221) | 0.001 | 1.747 (1.165-2.620) | 0.007 |
| Etiology (viral vs. non-viral) | 1.994 (1.210-3.286) | 0.007 | 1.509 (0.784-2.903) | 0.218 |
| Liver cirrhosis (yes vs. no) | 1.318 (0.988-1.758) | 0.060 | 1.043 (0.702-1.550) | 0.834 |
| HOXD10 expression (low vs high) | 1.594 (1.059-2.398) | 0.025 | 2.184 (1.135-4.202) | 0.019 |

HR: Hazard ratio; CI: confidence interval; TIL: tumor infiltrating lymphocytes; AJCC: American Joint Committee on Cancer; BCLC: Barcelona Clinic Liver Cancer; AFP: α-fetoprotein
[a]Partial data were not available, and statistics were based on the available data

**Table 4.** Multivariate analysis for recurrence free survival and disease specific survival

| Variables | *Recurrence free survival* | | *Disease specific survival* | |
|---|---|---|---|---|
| | *HR (95% CI)* | *p value* | *HR (95% CI)* | *p value* |
| Age (>55 yr vs. ≤55 yr) | 0.920 (0.630-1.343) | 0.665 | 1.200 (0.735-1.960) | 0.466 |
| Sex (male vs. female) | 1.487 (0.890-2.482) | 0.129 | 1.305 (0.701-2.600) | 0.369 |
| Microvascular invasion (yes vs. no) | 0.875 (0.571-1.340) | 0.540 | 0.695 (0.364-1.324) | 0.268 |
| Major portal vein invasion (yes vs. no) | 0.836 (0.395-1.769) | 0.639 | 1.465 (0.657-3.266) | 0.351 |
| Intrahepatic metastasis (yes vs. no) | 4.204 (2.700-6.544) | <0.001 | 4.976 (2.827-8.757) | <0.001 |
| Necrosis (yes vs. no) | 2.255 (1.490-3.415) | <0.001 | 4.000 (2.393-6.687) | <0.001 |
| TIL (no vs. yes) | 1.225 (0.861-1.743) | 0.260 | 1.846 (1.053-3.238) | 0.032 |
| Albumin level (≤3.5 g/dL vs. >3.5 g/dL) | 0.755 (0.453-1.257) | 0.280 | 0.782 (0.407-1.503) | 0.461 |
| AFP level[a] (>200 ng/mL vs. ≤200 ng/mL) | 1.387 (1.002-1.922) | 0.049 | 1.119 (0.700-1.791) | 0.638 |
| Etiology (viral vs. non-viral) | 1.294 (0.757-2.214) | 0.346 | | |
| Liver cirrhosis (yes vs. no) | 1.373 (1.001-1.884) | 0.049 | | |
| HOXD10 expression (low vs high) | 1.873 (1.193-2.938) | 0.006 | 2.504 (1.222-5.132) | 0.012 |

HR: Hazard ratio; CI: confidence interval; TIL: tumor infiltrating lymphocytes; AFP: α-fetoprotein
[a]Partial data were not available, and statistics were based on the available data.

Multivariate analysis results (Table 4) revealed that intrahepatic metastasis (p<0.001), necrosis (p<0.001), high serum AFP levels (p=0.049), and liver cirrhosis (p=0.049) were independent predictors of shorter RFS, while intrahepatic metastasis (p<0.001), necrosis (p<0.001), and low TIL levels (p=0.032) were independent predictors of shorter DSS. Low *HOXD10* expression was also an independent predictive factor for an unfavorable prognosis, showing statistical significance for reduction in both RFS (hazard ratio (HR) 1.873 (95% CI: 1.193-2.938), p=0.006) and DSS (HR 2.504 (95% CI: 1.222-5.132, p=0.012).

## Discussion

In the present study we showed that 82.7% of HCC samples presented with low HOXD10 expression. This low expression was associated with increased tumor size, advanced AJCC T and BCLC stages, and frequent early recurrence. In addition, our data showed that *HOXD10* expression was an independent predictive factor for early recurrence and an independent prognostic factor for RFS and DSS, respectively.

Homeobox genes encode a group of proteins that share a common homeodomain and act as transcription factors for their respective downstream targets [24]. Homeoproteins play an important role in carcinogenesis, as they are critical for cell growth, cycle regulation, migration, and death [10,11,25,26]. *HOXD10*, a member of the homeobox gene family, is known to suppress the transcription of genes associated with the remodeling of the extracellular matrix and endothelial cell migration, contributing to the maintenance of cellular differentiation [27]. *HOXD10* downregulation has been reported in various human cancers, and this downregulation is primarily controlled by changes in epigenetic methylation [14,16,17,28]. Experimental studies have suggested that *HOXD10* acts as a tumor suppressor in papillary thyroid carcinoma, colon cancer, endometrial carcinoma, pancreatic cancer, and prostate cancer [12-16]. The association between *HOXD10* expression and patient outcome has also been evaluated in several cancers, and increased expression has generally been linked to improved prognosis. In human cholangiocarcinoma, overall survival was significantly increased in HOXD10-positive patients [11]. In prostate cancer, tumor growth was significantly increased in a HOXD10-knockdown mouse xenograft model, and decreased *HOXD10* expression was an independent predictor of shorter RFS [12]. However, in head and neck squamous cell carcinoma, *HOXD10* expression was increased in primary tumor cells and decreased in lymph node metastatic cells, suggesting that it is dynamically regulated in response to tumor development and disease stage [29]. This suggests that *HOXD10* may have other functions in tumor development, and therefore, its role in different cancers should be evaluated.

To the best of our knowledge, only two previous studies have attempted to evaluate *HOXD10* expression in HCC. Zhou et al showed that *HOXD10* mRNA expression was downregulated in 18 HCC samples compared to their adjacent non-tumor tissues [27]. *HOXD10* overexpression increased apoptosis and inhibited the proliferative, migratory, and invasive properties of HCC cell lines; additionally, it suppressed tumor growth in a nude mouse xenograft model. Guo et al reported that *HOXD10* mRNA expression was regulated by promoter region methylation. *HOXD10* methylation was observed in 90 of the 117 HCC samples (76.9%) included in their study, which was associated with vessel cancerous emboli, differentiation, and patient survival [17]. In addition, this study confirmed that *HOXD10* acts as a tumor suppressor in both *in vitro* and *in vivo* xenograft models, and that *HOXD10* interacts directly with *IGFBP3*, suggesting that it is involved in extracellular signal-regulated kinase signaling [17]. In the current study, we used IHC to demonstrate that reduced *HOXD10* protein expression was closely related to frequent early recurrence rates and shorter RFS and DSS in a large cohort of HCC patients with long-term follow-up. These results are consistent with those of previous studies and provide clinical evidence regarding the utility of *HOXD10* as a prognostic biomarker and potential therapeutic target in HCC.

## Conclusion

Low *HOXD10* expression is common in HCC samples, and its expression is an independent prognostic factor for both RFS and DSS in curatively resected HCC. Our results suggest that *HOXD10* expression can be used as a prognostic biomarker and therapeutic target for HCC in the future.

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## Conflict of interests

The authors declare no conflict of interests.

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