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ORIGINAL RESEARCH

The Clinical Characteristics, Patterns of Recurrence, and Long-Term Survival Outcomes of Dual-Phenotype Hepatocellular Carcinoma After Curative Liver Resection

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Background & Aims: Dual-phenotype hepatocellular carcinoma (DPHCC) is discernible from classical HCC (CHCC) in its morphology and is characterized by the co-expression of both CHCC and cholangiocyte markers. This study aimed to clarify the difference between DPHCC and CHCC after surgery.

Methods: Patients with HCC after surgery were collected. The clinical characteristics, patterns of recurrence, and survival outcomes of patients with DPHCC and CHCC were compared. Multivariate analyses were used to determine the independent risk factors that influence the prognosis of patients.

Results: Patients with DPHCC (n = 141) account for 26% of the total patients (n = 541). Compared to patients with CHCC, patients with DPHCC are significantly associated with incomplete capsules, microvascular invasion, and poor differentiation (all $P < 0.05$). Compared to patients with CHCC, the 5-year overall survival (OS) (56% vs 43%) and recurrence-free survival (RFS) (35% vs 28%) are lower in patients with DPHCC. Meanwhile, among patients with tumor recurrence after surgery, patients with DPHCC have a higher proportion of advanced-stage tumors, and extrahepatic metastasis (all $P < 0.05$). Moreover, multivariate analysis showed that DPHCC is an independent risk factor for both OS (HR 1.399, 95% CI 1.061–1.845, $P = 0.017$) and RFS (HR 1.313, 95% CI 1.033–1.669, $P = 0.026$).

Conclusion: DPHCC, an aggressive HCC subtype with poor differentiation and high invasiveness, shows inferior RFS and OS post-liver resection compared to CHCC. Clinicians' recognition and addressing of its unique challenges can improve DPHCC patients' prognosis and QoL.

Keywords: hepatocellular carcinoma, dual-phenotype, recurrence, overall survival, prognosis

Introduction

Hepatocellular carcinoma (HCC), the preeminent primary malignancy of the liver, stands as a paramount contributor to cancer-associated mortality worldwide.[1,2] Despite liver resection being the cornerstone of curative therapy for patients with HCC, long-term outcomes remain unsatisfactory, plagued by a persistently elevated recurrence rate.[3] The substantial interpatient variability in prognosis among HCC cohorts is postulated to intimately intertwine with the inherent

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heterogeneity of these tumors, which encompasses a broad spectrum of pathological attributes and clinical presentations.[4,5] The hallmark of HCC, its heterogeneity, manifests in diverse clinical scenarios, underscoring the complexity of the disease.[6] Furthermore, this heterogeneity fosters the expression of cholangiocyte markers in select contexts, underscoring the intricate interplay between tumor biology and clinical behavior.[7]

Dual-phenotype hepatocellular carcinoma (DPHCC) was first described in 2011, characterized by the presence of both classical HCC (CHCC) markers (hepatocyte, glypican-3 (GPC-3), Arginase-1, and glutamine synthetase (GS)) and cholangiocyte markers (cytokeratin 7 (CK7), and cytokeratin 19 (CK19)).[8] Prior investigations into DPHCC have been predominantly centered on cellular and molecular biological aspects.[9,10] However, there persists a pressing need for a more exhaustive interrogation of DPHCC's distinct clinical presentations, biological behaviors, and long-term prognostic implications, when compared to patients with CHCC. As the research landscape of HCC evolves and deepens, gaining a nuanced understanding of these subtypes becomes paramount. Consequently, delving into DPHCC serves as a fundamental cornerstone for the precision tailoring of therapeutic and management strategies, aimed at achieving personalized outcomes for individual patients with HCC. This endeavor holds promise for advancing the overall management of this complex and heterogeneous malignancy. Thus, this study aimed to clarify the difference in clinical characteristics, patterns of recurrence, and long-term survival outcomes between CHCC and DPHCC after curative liver resection.

Methods

Patient Inclusion

Consecutive patients diagnosed with HCC underwent curative liver resection (R0) were collected between Jan. 2013 and Sep. 2020 at Zhejiang Provincial People's Hospital. The definitive diagnosis of patients afflicted with DPHCC and CHCC was achieved through rigorous pathological examination of the tissue specimens. Criteria for DPHCC diagnosis: (1) Tumor cell morphology consistent with HCC, (2) Expression of at least one cholangiocyte marker (such as CK7, or CK19) and at least one hepatocellular marker (Hepatocyte, GPC-3, Arginase 1, or GS) in over 15% of tumor cells simultaneously. Pathological and immunohistochemical findings were independently assessed and reported by two pathologists. The following exclusion criteria were utilized in the study: 1) Individuals aged below 18 or above 80 years, 2) Patients who had undergone any antitumor treatment either before or after hepatectomy, 3) Patients with recurrent tumors, macrovascular invasion, or other malignant conditions, 4) Cases of mixed intrahepatic cholangiocarcinoma (ICC) and HCC noted separately to avoid confusion, 5) Presence of extrahepatic metastasis, 6) Existence of other malignant tumors, 7) Patients suffering from severe cardiovascular or chronic pulmonary diseases, 8) Occurrence of perioperative mortality, 9) Death due to recurrence or death within 30 days post-surgery, and 10) Patients lacking complete medical records. This research adhered to the guidelines outlined by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) framework. Prior to undergoing surgery, all participating patients granted their informed consent. The conduct of this study was steered by the principles laid out in the Declaration of Helsinki and received the endorsement of the Ethics Committee at Zhejiang Provincial People's Hospital.

Liver Resection, Data Collection and Follow-up

Prior to surgery, all patients underwent comprehensive discussions among a multidisciplinary team. The determination of resectability for HCC hinges on various factors, namely liver functionality, tumor localization, and the estimated remaining liver volume post-surgery. Both traditional open surgery and laparoscopy surgery, which have been documented elsewhere[11], were employed as surgical approaches. We retrospectively gathered clinical and oncological data from patients' records. The baseline characteristics encompass gender, age, performance status, blood levels of aspartate transaminase (AST) and alanine aminotransferase (ALT), underlying liver disease cause (HBV versus others), presence of cirrhosis, Child-Pugh classification, preoperative levels of alpha-fetoprotein (AFP) and carbohydrate antigen 19–9 (CA 19–9), tumor size, number of tumors, tumor encapsulation, differentiation, resection margin, extent of hepatectomy, intraoperative blood loss and transfusion, and surgery duration. Post-hepatectomy, patients were monitored every 2 months for the initial 2 years, followed by check-ups every 3 to 6

months thereafter. During each follow-up, serum tumor markers (AFP and CA19-9) and abdominal ultrasound were evaluated. Additionally, every 3 months, an enhanced computed tomography scan or magnetic resonance imaging was conducted to detect any signs of HCC metastasis or recurrence. The latest follow-up was completed in December 2023. Recurrence-free survival (RFS) was calculated from the surgery date until tumor recurrence was observed. Overall survival (OS) was measured from the surgery date to the last follow-up visit or the patient's demise. Management strategies for recurrent tumors were decided through multidisciplinary discussions at each center. The treatment options ranged from curative therapies, including local radiofrequency ablation, liver transplantation, and re-resection, to non-curative therapies such as transarterial chemoembolization (TACE), radiotherapy, systemic therapy involving chemotherapy, targeted therapy, or immunotherapy, and best supportive care, either alone or in combination.

Statistical methods

In accordance with prior research findings and clinical relevance, continuous variables were transformed into categorical variables. To illustrate the distribution of each categorical variable, frequencies and percentages were employed. Differences amongst these variables were assessed using either Pearson's chi-square test or Fisher's exact test, depending on their suitability. Kaplan–Meier survival curves facilitated the estimation of OS and RFS within each cohort, with comparisons drawn through Log rank tests. Variables identified to be significant at the level of $P < 0.1$ in the univariate analysis were subsequently included in a forward stepwise multivariate Cox proportional hazard regression model. Statistical significance was determined with a threshold p-value of <0.05. The statistical computations for this study were executed using R software, version 4.3.2, accessible at http://www.r-project.org/.

Results

Clinical Characteristics

A total of 541 patients were finally included in this study, comprising 400 (74%) patients with CHCC and 141 (26%) patients with DPHCC, respectively (Table 1). Most of the patients were male (n = 458, 84.6%), with HBV infection (n = 458, 84.6%) and cirrhosis (n = 399, 73.7%). Compared to patients with CHCC, the proportion of MVI (83% vs 69%), incomplete capsule (53.0% vs 41.5%), and poor differentiation (38.3% vs 21.5%) are most presented in patients with

Table 1 Comparison of Clinical Characteristics Between the Two Groups Stratified by Pathological Markers for Patients with Hepatocellular Carcinoma After Curative Liver Resection

Variables (n, %)	All Patients (n = 541)	DPHCC Group (n = 141)	CHCC Group (n = 400)	P
Sex, male	458 (84.7)	118 (83.7)	340 (85.0)	0.710
Age > 65 years	126 (23.3)	31 (22.0)	95 (23.8)	0.670
Performance status, ≥ 1	141 (26.1)	43 (30.7)	98 (24.5)	0.150
Etiology of liver disease, HBV	458 (84.7)	124 (87.9)	334 (83.5)	0.208
Cirrhosis, yes	399 (73.8))	106 (75.2)	293 (73.3)	0.655
Child-Pugh grade, B	47 (8.7)	12 (8.5)	35 (8.8)	0.931
Preoperative AST level, > 40 U/L	220 (40.7)	54 (38.3)	166 (41.5)	0.506
Preoperative ALT level, > 40 U/L	168 (31.1)	36 (25.5)	132 (33.0)	0.099
AFP, > 20 ng/L	297 (54.9)	82 (58.2)	215 (53.8)	0.366
CA19-9, > 37 ug/L	97 (17.9)	32 (22.7)	65 (16.3)	0.058
Maximum tumor size, ≥ 5 cm	179 (33.1)	46 (32.6)	133 (33.3)	0.892
Tumor numbers, multiple	98 (18.1)	27 (19.1)	71 (17.8)	0.711
Incomplete capsule, yes	393 (72.6)	117 (83.0)	276 (69.0)	0.001
Satellites, with	49 (9.1)	13 (9.2)	36 (9.0)	0.938

(*Continued*)

Table 1 (Continued).

Variables (n, %)	All Patients (n = 541)	DPHCC Group (n = 141)	CHCC Group (n = 400)	P
Microvascular invasion	241 (44.5)	75 (53.2)	166 (41.5)	0.016
Tumor differentiation, poor	140 (25.9)	54 (38.3)	86 (21.5)	<0.001
Resection margin, < 1 cm	147 (27.2)	39 (27.7)	108 (27.0)	0.880
Major hepatectomy, yes	123 (22.7)	33 (23.4)	90 (22.5)	0.826
Intraoperative blood loss, > 600 mL	115 (21.3)	37 (26.2)	78 (19.5)	0.093
Blood transfusion, yes	136 (25.1)	30 (21.3)	106 (26.5)	0.219
Operation time, > 180 min	294 (54.3)	81 (57.4)	213 (53.3)	0.390

Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate transaminase; AFP, alpha-fetoprotein; CA 19–9, Carbohydrate antigen 19–9.

DPHCC (all P < 0.05). There were no significant correlations between DPHCC and CHCC groups regarding Child-Pugh stage, tumor size, and tumor numbers (all P > 0.05). In addition, although there was no statistically significant difference in CA19-9 level between the two groups, a higher proportion of patients in CA19-9 >37 ug/L (22.7% vs 16.3%, P = 0.058) was found in the DPHCC group.

Survival Outcomes, Patterns of Recurrence, and Treatment Modalities

Following a median tracking duration of 71.0 months, 67.1% of patients, totaling 363, experienced tumor recurrence, while 49.7% of patients, equivalent to 269 individuals, either succumbed to the disease or were unavailable for further follow-up. When contrasting patients with CHCC to those with DPHCC, it is evident that the 5-year OS rate for DPHCC patients stands at 43%, significantly lower than the 56% observed in CHCC patients (P = 0.003). Similarly, the 5-year RFS rate for DPHCC patients is 28%, notably decreased compared to the 35% seen in CHCC patients (P = 0.006) (depicted in Figure 1a and b). Among the recurrent cases, 263 belonged to the CHCC group, and 100 were from the DPHCC group. Table 2 displays a comparative analysis of the tumor stage at recurrence, recurrence patterns, and treatment approaches between CHCC and DPHCC patients. The findings indicate that, in comparison to CHCC patients, DPHCC patients exhibit a decreased incidence of early-stage tumors at recurrence (falling within Milan Criteria: 46.4% vs 53.6%, P = 0.047; and BCLC stage A: 21.0% vs 36.1%, P = 0.044). Conversely, DPHCC patients have a higher frequency of extrahepatic metastases (13.0% vs 6.1%, P = 0.029) and a reduced likelihood of receiving curative treatment (40.0% vs 55.5%, P = 0.006).

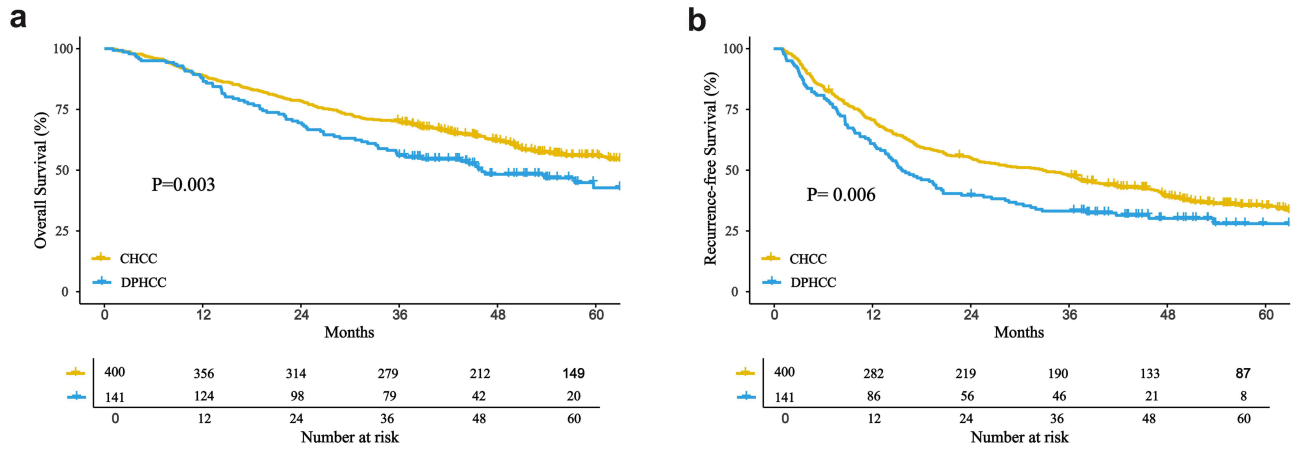

Figure 1 The K-M curves comparisons of overall survival and recurrence free survival between patients with DPHCC and CHCC. (**a**) Overall survival, (**b**) Recurrence-free survival.

Abbreviations: DPHCC, Dual-phenotype hepatocellular carcinoma; CHCC, classical hepatocellular carcinoma.

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Table 2 Comparison of Survival Outcomes, Patterns of Recurrence, and Treatment Modalities Between the Two Groups Stratified by Pathological Markers for Patients with Hepatocellular Carcinoma After Curative Liver Resection

Variables	All Patients (n = 541)	DPHCC Group (n = 141)	CHCC Group (n = 400)	P
Overall survival (OS)				
Median OS (months)	65	46	71	0.012
1-year OS	87%	86%	88%	0.003
3-year OS	66%	55%	69%	
5-year OS	53%	43%	56%	
Number of death (%)	269 (49.7)	78 (55.3)	191 (47.8)	0.074
Recurrence-free survival (RFS)				
Median RFS (months)	25	15	32	0.001
1-year RFS	66%	59%	68%	0.006
3-year RFS	44%	33%	47%	
5-year RFS	33%	28%	35%	
Number of recurrences (%)	363 (67.1)	100 (70.9)	263 (48.6)	0.154
Milan criteria at recurrence				0.047
Within Milan (%)	158 (43.5)	36 (36.0)	122 (46.4)	
Beyond Milan (%)	205 (56.5)	64 (64.0)	141 (53.6)	
BCLC stage at recurrence				0.044
A (%)	116 (32.0)	21 (21.0)	95 (36.1)	
B (%)	70 (19.3)	22 (22.0)	48 (18.3)	
C (%)	130 (35.8)	41 (41.0)	89 (33.8)	
D (%)	47 (12.9)	16 (16.0)	31 (11.8)	
Patterns of recurrence				0.029
Intrahepatic recurrence only (%)	334 (92.0)	87 (87.0)	247 (93.9)	
Extrahepatic metastasis (%)	29 (8.0)	13 (13.0)	16 (6.1)	
Treatment modality				0.006
Curative treatment (%)	186 (51.2)	40 (40.0)	146 (55.5)	0.010
Hepatectomy (%)	154 (82.7)	33 (82.5)	121 (82.9)	1.000
Radiofrequency ablation (%)	32 (17.2)	7 (17.5)	25 (17.1)	1.000
Noncurative treatment (%)	177 (48.8)	60 (60.0)	117 (44.5)	0.010
TACE* (%)	93 (52.5)	34 (56.7)	59 (50.4)	0.525
Systemic therapy (%)	37 (20.9)	11 (18.3)	26 (22.2)	0.697
Best supportive treatment (%)	47 (26.6)	15 (25.0)	32 (27.4)	0.858

Note: *including TACE with or without radiotherapy and/or systemic therapy.
Abbreviations: OS, Overall survival; RFS, Recurrence-free survival; BCLC, Barcelona Clinic for Liver Cancer; TACE, transcatheter arterial chemoembolization.

Independent Risk Factors of OS and RFS

The outcomes derived from multivariable Cox regression analyses indicate that DPHCC stands as a significant and autonomous predictor for both OS (HR = 1.399; 95% CI: 1.061–1.845, P = 0.017) and RFS (HR = 1.313; 95% CI: 1.033–1.669, P = 0.026), as presented in Tables 3 and 4. Furthermore, the presence of AFP levels exceeding 20 ng/L, multiple tumors, an incompletely encapsulated state, satellite nodules, microvascular invasion, and a resection margin narrower than 1cm are each identified as distinct risk factors that independently influence both OS and RFS. Notably, a Child-Pugh grade of B solely emerges as an independent risk factor for OS.

Discussion

This study was designed to systematically contrast the clinical characteristics, recurrence patterns, and survival outcomes between patients diagnosed with DPHCC and CHCC. A comprehensive analysis encompassed 541 patients, among whom 141 were confirmed with DPHCC. Baseline characteristics underscored the propensity for patients with DPHCC

Table 3 Univariable and Multivariable Cox-Regression Analyses of Risk Factors Associated with Overall Survival for Patients with Hepatocellular Carcinoma After Curative Liver Resection

Variables	UV HR (95% CI)	P	MV HR (95% CI)	P*
Sex, male	0.886 (0.639–1.227)	0.465		
Age > 65 years	1.120 (0.848–1.480)	0.424		
Performance status, ≥ 1	1.319 (0.808–2.975)	0.234		
Etiology of liver disease, HBV	0.948 (0.686–1.310)	0.746		
Cirrhosis, yes	1.145 (0.867–1.512)	0.339		
Child-Pugh grade, B	1.882 (1.307–2.710)	0.001	1.487(1.004–2.201)	0.048
Preoperative AST level, > 40 U/L	1.524 (0.857–2.190)	0.169		
Preoperative ALT level, > 40 U/L	1.301(0.813–1.671)	0.137		
AFP, > 20 ng/L	1.406 (1.101–1.794)	0.006	1.394 (1.040–1.867)	0.026
CA19-9, > 37 ug/L	1.724 (1.311–2.268)	<0.001	NS	
Maximum tumor size, ≥ 5 cm	2.455 (1.930–3.122)	<0.001	NS	
Tumor numbers, multiple	1.696 (1.278–2.250)	<0.001	1.135 (1.061–1.496)	0.038
Incomplete capsule, yes	2.096 (1.556–2.822)	<0.001	1.498 (1.240–2.326)	0.001
Satellites, with	2.423 (1.709–3.434)	<0.001	1.588 (1.078–2.338)	0.019
Microvascular invasion	2.648 (2.050–3.419)	<0.001	2.196 (1.594–3.026)	<0.001
Tumor differentiation, poor	1.411 (0.884–1.837)	0.210		
Resection margin, < 1 cm	1.731 (1.342–2.233)	<0.001	1.665 (1.278–2.170)	<0.001
Major hepatectomy, yes	1.282 (0.706–1.366)	0.149		
Intraoperative blood loss, > 600 mL	1.183 (0.903–1.549)	0.223		
Blood transfusion, yes	1.146 (0.848–1.549)	0.375		
Operation time, > 180 min	1.389 (0.622–1.772)	0.198		
DPHCC, yes	1.495 (1.145–1.952)	0.003	1.399(1.061–1.845)	0.017

Note: *P < 0.1 in univariable analyses were entered into multivariable Cox analyses.
Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate transaminase; AFP, alpha-fetoprotein; CA 19–9, Carbohydrate antigen 19–9; UV, univariable; MV, multivariable; NA, not available; HR, hazard ratio; DPHCC, Dual-phenotype hepatocellular carcinoma; NS, no significance.

Table 4 Univariable and Multivariable Cox-Regression Analyses of Risk Factors Associated with Recurrence-Free Survival for Patients with Hepatocellular Carcinoma After Curative Liver Resection

Variables	UV HR (95% CI)	P	MV HR (95% CI)	P*
Sex, male	1.123 (0.837–1.507)	0.438		
Age > 65 years	1.058 (0.833–1.344)	0.644		
Performance status, ≥ 1	1.208 (0.901–1.620)	0.206		
Etiology of liver disease, HBV	1.125 (0.842–1.502)	0.425		
Cirrhosis, yes	1.288 (0.812–1.639)	0.440		
Child-Pugh grade, B	1.314 (0.928–1.863)	0.124		
Preoperative AST level, > 40 U/L	1.058 (0.806–1.389)	0.684		
Preoperative ALT level, > 40 U/L	1.518 (0.824–1.883)	0.501		
AFP, > 20 ng/L	1.288 (0.946–1.586)	0.117	1.101 (1.092–1.822)	0.008
CA19-9, > 37 ug/L	1.688 (1.325–2.152)	<0.001	NS	
Maximum tumor size, ≥ 5 cm	2.206 (1.386–2.725)	0.008	NS	
Tumor numbers, multiple	2.043 (1.598–2.612)	<0.001	1.702 (1.320–2.195)	<0.001
Incomplete capsule, yes	1.762 (1.379–2.250)	<0.001	1.504 (1.159–1.951)	0.002
Satellites, with	2.387 (1.732–3.289)	<0.001	1.426 (1.003–2.026)	0.048
Microvascular invasion	1.856 (1.507–2.285)	<0.001	1.127 (1.086–1.433)	0.030
Tumor differentiation, poor	1.336 (1.061–1.638)	0.014	NS	

(*Continued*)

Table 4 (Continued).

Variables	UV HR (95% CI)	P	MV HR (95% CI)	P*
Resection margin, < 1 cm	1.637 (1.311–2.044)	<0.001	1.459 (1.159–1.836)	0.001
Major hepatectomy, yes	1.491 (0.503–2.379)	0.241		
Intraoperative blood loss, > 600 mL	1.144 (0.698–2.708)	0.301		
Blood transfusion, yes	1.038 (0.827–1.303)	0.746		
Operation time, > 180 min	1.361 (0.804–1.676)	0.204		
DPHCC, yes	1.377 (1.092–1.737)	0.007	1.313 (1.033–1.669)	0.026

Note: * P < 0.1 in univariable analyses were entered into multivariable Cox analyses.
Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate transaminase; AFP, alpha-fetoprotein; CA 19–9, Carbohydrate antigen 19–9; UV, univariable; MV, multivariable; NA, not available; HR, hazard ratio; DPHCC, Dual-phenotype hepatocellular carcinoma; NS, no significance.

to manifest MVI and incomplete capsules, compared to patients with CHCC. Additionally, the tumor cells in patients with DPHCC exhibited a lower degree of differentiation. Notably, patients with DPHCC have been demonstrated a heightened postoperative recurrence rate, with recurrent tumors tending to manifest at intermediate to advanced stages, consequently diminishing the feasibility of radical therapeutic interventions. These observations collectively suggest a heightened tumor aggressiveness inherent to DPHCC. Multivariate Cox proportional hazards regression analysis fortified this notion, identifying DPHCC as an autonomous predictor of both OS (HR 1.399) and RFS (HR 1.313). In other words, patients with DPHCC confronted a roughly 1.3-fold augmented risk of both recurrence and mortality, in comparison to patients with CHCC.

The origin of HCC has been a subject of ongoing debate. One prevailing hypothesis posits that HCC arises from the abnormal differentiation of hepatic progenitor cells (HPCs), encompassing both HCC and ICC.[12] HPCs, also known as oval cells, were first discovered by Farber in 1956, when he established an animal model of induced tumorigenesis using carcinogenic agents. Under normal circumstances, HPCs remain in a relatively quiescent state. However, in the presence of persistent injury that impedes the proliferation of normal hepatocytes or cholangiocytes, HPCs can be activated and differentiate into mature hepatocytes and cholangiocytes to participate in liver repair.[13] Tumorigenesis is also considered a process of unresolved injury repair. Exposure to adverse environments, such as reactive oxygen species (ROS) and chronic inflammation, inevitably involves the activation of HPCs. These risk factors often contribute to tumorigenesis, and thus, the malignant transformation of HPCs can also underlie the development of liver cancer. This aligns with the "maturation arrest theory" of tumor origin, which posits that HCC arises from the blocked maturation and subsequent malignant transformation of HPCs.[14] In essence, the formation of liver cancer, including HCC and ICC, is a process where the mature differentiation of progenitor cells is hindered, leading to their transformation into cancerous cells. Thus, DPHCC might be linked to HPC, which exhibit cholangiocyte markers like CK7 and CK19 in patients with HCC.[15,16] Distinguished from mixed HCC and ICC, DPHCC is regarded as a specific condition of abnormal differentiation of HPCs, in which a single tumor cell expresses both HCC markers and ICC markers.[17]

Before the nomenclature of DPHCC, earlier studies reported that HCC patients expressing cholangiocyte markers were associated with early postoperative recurrence and higher mortality risk.[18,19] Since Lu et al defined HCC expressing both hepatocytic and cholangiocyte markers as DPHCC in 2011,[8] subsequent research on DPHCC has gradually expanded. Some studies have indicated that DPHCC may be associated with a poorer prognosis, which is consistent with our findings.[8,20] Additionally, reports suggest that the imaging characteristics of DPHCC fall between those of HCC and ICC, showing a greater tendency for arterial phase ring enhancement and infiltrative features. These imaging characteristics suggest that DPHCC may possess greater aggressiveness, which aligns with our results.[21,22] The aggressive behavior and worse prognosis of DPHCC may be associated with its specific gene mutations and the expression of molecular markers. A comprehensive genomic and transcriptomic analysis of DPHCC suggests that it has a unique gene mutation profile, which is related to the invasiveness and proliferation of cancer cells.[23–25] Furthermore, DPHCC can express cholangiocyte markers such as CK7 and CK19, which provides further insight into its characteristics. Earlier research has identified CK19 as a marker for HPC, suggesting increased malignancy,[26] thus indicating that DPHCC might be less differentiated. In

addition, CK19 enhances blood vessel formation, increases invasiveness, and correlates with increased rates of recurrence after surgery and reduced survival rates.[26–29] Elevated CK7 levels in cancer cells have also been associated with the carcinogenic properties, invasiveness, and metastatic potential of hepatocellular carcinoma.[30]

The results of our study carry profound implications for clinical practice. The observed heightened aggressiveness, decreased differentiation, and shortened RFS and OS of DPHCC in comparison to CHCC suggest that DPHCC should be acknowledged as a distinct subset within the broader category of HCC. Recognizing DPHCC as such is pivotal for enhancing patient prognosis and directing clinical decision-making processes. Given DPHCC's aggressive characteristics, a more vigilant and frequent monitoring approach is imperative for patients diagnosed with this subtype. As DPHCC progresses rapidly and has a higher propensity for recurrence, clinicians should consider adopting more rigorous follow-up protocols to swiftly detect and manage recurrences. Furthermore, DPHCC's reduced differentiation indicates potential resistance to standard therapies, emphasizing the necessity to explore novel therapeutic strategies and conduct clinical trials specifically tailored for DPHCC. Tailored treatment plans, formulated based on DPHCC's molecular profile, could potentially elevate overall survival rates.

Certain considerations must be taken into account when interpreting the results of this study. Being retrospective in nature, standardization and identification of certain factors were inherently challenging. Furthermore, the patient cohort was predominantly Chinese, with over 80% having a history of HBV infection. The applicability of these findings to HCV-related HCC remains uncertain and warrants further research. Moreover, in non-randomized controlled trials (RCTs), perioperative mortality can introduce bias into the perceived efficacy of adjuvant treatments. To mitigate this, we excluded patients who succumbed within 30 days of liver resection. However, it is crucial to acknowledge that this exclusion might introduce another type of bias stemming from conditional survival, where patients who have already survived a certain period post-treatment inherently exhibit improved prognosis.[31] Thus, a multicenter RCT remains essential to conclusively establish the efficacy of the treatments. Additionally, there is a need to clearly define the role of TACE as a monotherapy or in combination with other relevant treatments.

Conclusion

Our study's findings underscore DPHCC's unique aggressiveness, poor differentiation, and reduced RFS/OS, distinct from CHCC, highlighting its importance as a separate HCC subtype. This distinction is vital for clinical management, requiring heightened surveillance and more frequent monitoring. DPHCC's aggressive nature and recurrence potential warrant intensive follow-up to detect recurrences promptly. Resistance to conventional therapies underscores the need for novel therapeutics and tailored trials. Personalized, precision treatments tailored to DPHCC's molecular profile may enhance OS. Shorter survival underscores the urgency for individualized strategies. Recognizing DPHCC's unique challenges optimizes management, enhancing prognosis and patient quality of life.

Abbreviations

HCC, hepatocellular carcinoma; DPHCC, Dual-phenotype hepatocellular carcinoma; CHCC, classical hepatocellular carcinoma; CK19, Cytokeratin 19; CK7, Cytokeratin 7; GPC-3, Glypican-3; GS, Glutamine synthetase; HR, hazard ratio; CI, confidence interval; RFS, Recurrence-free survival; OS, Overall survival.

Data Sharing Statement

Upon reasonable request, the corresponding author can provide access to the datasets employed and examined in the ongoing research.

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Zi-Chen Yu, Zheng-Kang Fang and Yang Yu contributed equally to this work.

Author Contributions

Each author has played a crucial role in the research presented, encompassing the conceptualization, study design, execution, data acquisition, analysis, and interpretation, among other areas. They have all actively participated in

drafting, revising, and critically reviewing the article. Furthermore, they have approved the final version for publication, agreed upon the journal for submission, and accepted responsibility for all facets of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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