

^{31}P Magnetic Resonance Spectroscopic Imaging Reflects Metabolic Features of Hepatocellular Carcinoma and Their Correlations with Tumor Aggressiveness

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Abstract

Purpose

To investigate whether ^{31}P two-dimensional magnetic resonance spectroscopic imaging (^{31}P -2D-MRSI) could noninvasively reflect HCC metabolic features and to evaluate their correlation with tumor aggressiveness.

Methods

This prospective study classified patients with HCC into early-stage [Barcelona Clinic Liver Cancer (BCLC) 0/A] and advanced-stage (BCLC B/C). Phospholipid metabolism ratios were obtained from lesion and background voxels using ^{31}P -2D-MRSI, respectively. Patients were further stratified by Ki-67 expression (early-stage) or treatment-response to chemotherapy (advanced-stage). Significant differences in the ratios were assessed using independent-samples t test or Mann–Whitney U test.

Results

When comparing all HCC lesions ($n = 44$) with background tissue ($n = 37$) and, within lesions, advanced-stage ($n = 21$) with early-stage tumors ($n = 23$), both analyses showed higher mean PME/PDE (0.91 vs 0.50; 1.41 vs 0.71, all $p < 0.017$) and PE/PDE (0.50 vs 0.28; 0.73 vs 0.40) ratios in lesions and advanced-stage tumors. In early-stage HCCs, high Ki-67 expression lesions ($> 20\%$, $n = 10$) exhibited higher mean PME/PDE and PE/PDE (0.73 vs. 0.51; 0.44 vs. 0.27) ratios than low Ki-67 expression lesions ($\leq 20\%$, $n = 13$). However, within advanced-stage HCCs, non-responding lesions ($n = 13$) showed lower mean PME/PDE and PE/PDE ratios (1.05 vs. 2.19; 0.63 vs. 1.02) than responding lesions ($n = 8$).

Conclusion

^{31}P -2D-MRSI may stratify HCC aggressiveness by metabolic ratios.

Introduction

Hepatocellular carcinoma (HCC) is responsible for a considerable number of cancer-related deaths [1]. According to the Barcelona Clinic Liver Cancer (BCLC) staging system, the median survival time of advanced-stage HCC is nearly 2.5 years, while that of early-stage HCC is over 5 years [2]. This difference is primarily attributed to various aggressive biological behaviors, such as differing levels of Ki-67 expression [2, 3]. Invasive pathological assessment remains as a current gold standard for evaluating tumor aggressiveness [4, 5]. However, these methods are constrained by their invasiveness and risk of procedure-related complications [5].

Metabolic reprogramming of phospholipid metabolism is a hallmark of HCC aggressiveness [6, 7]. Phosphorus-31 magnetic resonance spectroscopic imaging (^{31}P MRSI) is a noninvasive technique for assessing metabolic alterations by quantification of phosphorus-containing metabolites [8, 9]. ^{31}P MRSI

provides insight into membrane metabolism by detecting phosphomonoesters (PMEs) and phosphodiesteres (PDEs). PMEs, containing phosphoethanolamine (PE) and phosphocholine (PC), are involved in cell membrane synthesis, whereas PDEs, containing glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC), are products of cell membrane degradation [8, 9].

Previous reviews outlined that liver tumors exhibited an elevated PME/PDE ratio compared with healthy liver tissue, reflecting increased tumor cell proliferation [8, 10, 11]. However, few studies have concentrated on HCC [12, 13]. Therefore, metabolic characteristics of HCC remain not fully understood. Panda et al. [13] revealed an elevated PME/PDE ratio in the HCC voxel compared to the nonmalignant voxel in a study involving only one patient with HCC. Yuan et al. [12] monitored a significant decrease in PDE/total phosphorus content (TPC) ratio within the HCC after chemotherapy compared to the baseline. However, little work has been done to explore the relationship between metabolic alterations and tumor aggressiveness due to the absence of detailed pathological assessment of HCC.

We hypothesize that metabolic data measured by ^{31}P MRSI could profile the metabolic features of HCC compared to the surrounding liver tissue, and that these features may vary across different lesions with varying levels of aggressiveness. Therefore, this study aimed to (i) employ phosphorus-31 two-dimensional magnetic resonance spectroscopic imaging (^{31}P 2D MRSI) to characterize the metabolic profiles of HCC relative to the surrounding liver, and (ii) investigate the relationship between metabolic indices and tumor aggressiveness through subgroup analyses stratified by BCLC stage, tumor Ki-67 expression, and response to hepatic arterial infusion chemotherapy (HAIC).

Materials and Methods

This study was approved by the Institutional Review Board (No. xx2022-266) on 15th November, 2022 and written informed consents were obtained from all participants. Between October 2023 and August 2024, 94 patients with a high suspicion of HCC were enrolled in this study and underwent ^{31}P 2D MRSI of the liver. The inclusion criteria were: (a) radiologically suspected HCC and (b) age between 18 and 80 years. The exclusion criteria were as follows: (a) the presence of other types of tumors, (b) previous treatment of HCC, and (c) suboptimal ^{31}P 2D MRSI quality for analysis ($\text{PCr} > \alpha\text{-ATP}$ or $\text{SNR} < 4$) [14]. Twenty-nine patients were excluded, and 65 were included in the final analysis (Fig. 1).

Acquisition and localization of ^{31}P 2D MRSI in the human liver

MR examinations were performed on a 3.0-T scanner (Elition; Philips Healthcare, Best, the Netherlands). Participants were scanned supine after at least 4-hour fasting. Initial localization was performed using transversal T2-weighted imaging [repetition time (TR) = 2000 milliseconds; echo time (TE) = 66 milliseconds (ms)], acquired with a torso coil.

A flexible ^{31}P transmit-receive loop surface coil (14 cm in diameter) was then positioned on the skin surface, directly over the lesions, replacing the torso coil. Precise surface coil position was confirmed by T1-weighted transversal and coronal images [TR/TE = 8/2.3 ms; flip angle = 15°; field of view (FOV) = 450 · 450 · 60 mm³], acquired with the ^1H body coil, which showed two high-signal-intensity spot markers at the center of the coil.

^{31}P 2D MR spectra were acquired by carefully placing the FOV parallel to the spot markers, ensuring that the lesion was as close to the center of FOV as possible. The shim volume was adjusted to reduce interference from the lungs, gallbladder, and abdominal muscles, while maximizing coverage of the target region. For patients with multiple lesions, generally those within the right liver and neither too superficial to avoid muscle contamination nor too deeply seated to fall outside the detection range of the surface coil were included for FOV placement to achieve an ideal signal-to-noise ratio (SNR), although this approach may inevitably introduce selection bias. The acquisition time of ^{31}P 2D MR spectra was accelerated from 8 to 4 minutes using *k*-space weighting. Broadband decoupling was performed using a wideband alternating-phase low-power technique with zero-residual splitting and 4 phase cycling.

After obtaining ^{31}P 2D MR spectra, another set of sagittal T1 weighted images with the same slice orientation with the spectra were acquired for reference. The total protocol duration was approximately 15 minutes per patient. Detailed imaging parameters of the image selected in vivo spectroscopy (ISIS) with slice selective ^{31}P 2D MRSI were listed in Supplementary Table 1[16].

^{31}P 2D MRSI data processing

Spectra were processed using the embedded post-processing software package SpectroView (Philips, Best, The Netherlands). Area of PE, PC, inorganic phosphate (Pi), GPE, GPC, phosphocreatine (PCr), nicotinamide adenine dinucleotide, γ -ATP, α -ATP, and β -ATP (not reported, because of the limited bandwidth of the excitation pulse) were fitted with an iterative nonlinear least-squares technique. The spectra were apodized with a Gaussian line shape of 12 Hz, manually phase corrected and frequency adjusted by setting the γ -ATP resonance to 2.40 ppm. Voxels with SNR less than 4 or contaminated by skeletal muscle (PCr > α -ATP) or gall bladder (PtdC > α -ATP) were excluded [15]. SNR was calculated by dividing the height of the γ -ATP peak by the standard deviation (SD) of the noise (taken from the upfield of PE and downfield of β -ATP) [16], and other details were listed in Supplementary Table 2.

One or two averaged spectra per ^{31}P 2D MRSI dataset were selected from voxels in the center of the FOV. The spectra were selected to correspond to HCC lesions or background tissue, respectively. This selection was confirmed by transversal T2-weighted and sagittal T1-weighted proton images. Extensive tumors, occupying four or more voxels in the center of FOV, were excluded from the background analysis, with only lesion voxels included for evaluation. Small lesions, not visible on sagittal images, and deep-seated lesions, located beyond the surface coil's sensitivity range (typically 7 cm or more from the skin surface), were excluded from lesion analysis, with only background voxels selected for analysis.

The analyst (___, with 2 years of experience in MRSI and MRI) independently performed voxel selection and spectral fitting, blinded to clinical and histopathological data. The fitting results were then reviewed by two senior researchers: an imaging expert with 11 years of MRI experience, who verified that the voxels were within the lesion, and a supervisor with 7 years of MRSI experience, who ensured that (a) the baseline was smooth enough to capture slow background variations, (b) the residuals appeared noise-like across the entire fitting region, (c) the fitted profile accurately captured the core and wings without obvious under- or overestimation, and (d) the positions of each fitted peak aligned with expected transitions [17–20].

The ^{31}P 2D MRSI results were expressed as relative ratios between single resonances or groups, such as the TPC [21]. Relative ratios reflect hepatic phosphorus metabolism changes, albeit that non-uniform coil sensitivity, field inhomogeneity, and relaxation time will affect these ratios, once metabolic levels are not constant within the true voxel area [22].

Clinical Information.

All patients were evaluated using the BCLC staging system [23]. The early-stage group includes patients with BCLC 0-A stage who underwent hepatic resection, and the advanced-stage group includes those with BCLC B-C stage who received HAIC, with or without subsequent therapy. Information on total bilirubin (TB), albumin (ALB), prothrombin time (PT), increased international normalized ratio (INR), prealbumin (PAB), platelet (PLT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) values was collected before treatment.

Ki-67 Index Measurement

The Ki-67 index was obtained directly from the medical records of ___ Hospital, evaluated by pathology experts. The Ki-67 index is calculated as the proportion of positive cells (brown-stained nuclei) to the total cell count. Higher Ki-67 (> 20%) expression are considered as a marker of more aggressive phenotypes of HCC based on previous literatures [5, 24] and the patients were stratified according to this value.

Tumor Response Assessment

Response evaluation followed the modified response evaluation criteria in solid tumors after completing HAIC (8 infusions) [25]. For patients undergoing early (within 8 infusions) conversion surgery, the response was assessed based on established histologic criteria [26]. Patients were categorized into a response group, which included complete response, partial response, pathologic complete response, and major pathologic response (MPR), or a nonresponse group, which comprised stable disease, progressive disease, or the absence of MPR. The nonresponsive lesions exhibit more aggressive behavior than the responsive lesions [26, 27].

Statistical analysis

Sample size calculations were performed using the G*Power program (version 3.1.9.7) [28]. Based on our pilot study, effect sizes of 1.54 and 1.94 were calculated, with matching ratios of 1:1 and 2:1, derived from the PME/PDE ratio between the low and high Ki-67 expression groups in the early-stage group, and between the response and nonresponse groups in the advanced-stage group, respectively. Using a two-tailed test with an alpha error of 0.017 and a power of 80% [29], the required sample sizes were calculated to be 22 cases for the early-stage group and 18 cases for advanced-stage group. However, to enhance the study's power, 23 patients were included in the early-stage group and 21 patients in the advanced-stage group.

Python 3.12.1 (www.python.org) was used for further statistical analysis. Normality was assessed using the Shapiro-Wilk test before comparisons. Differences between groups were evaluated using the appropriate statistical tests, including the independent-samples *t* test, Mann–Whitney *U* test, chi-square test, or Fisher's exact test. Data are presented as median with interquartile range (IQR) intervals (nonnormal distribution) or mean $\pm$ SD (normal distribution). Relative ratios from ^{31}P 2D MRSI outside $Q1 - 1.5 \cdot \text{IQR}$ or $Q3 + 1.5 \cdot \text{IQR}$ were considered as extreme values and excluded to ensure a more robust basis for analysis. A *p* value < 0.05 was used to indicate a significant difference between the background and lesion voxels, as the background voxels were not involved in further comparisons. Bonferroni corrections [30] were applied to adjust for multiple comparisons within the lesion subgroups, with a threshold of $p < 0.017$ considered statistically significant.

Results

Demographic and clinical characteristics of the study population

^{31}P 2D MRSI data from 65 patients (mean age: 59 ± 11 years; 56 men) were analyzed. In cases of extensive tumors ($n = 28$), only lesion voxels were acquired. Conversely, in patients with small tumors (< 10 mm, $n = 8$) or deep-seated lesions ($n = 13$), only background liver voxels were obtained. As a result, 44 lesion voxels and 37 background liver voxels were included.

Based on the BCLC staging system, the patients with lesion voxels, were categorized into either the early-stage (BCLC 0/A, $n = 23$) or advanced-stage (BCLC B/C, $n = 21$) group. Within the early-stage group, the 23 patients were further categorized into either the low Ki-67 ($\leq 20\%$, $n = 13$) or high Ki-67 expression ($> 20\%$, $n = 10$) group based on Ki-67 expression levels. In the advanced-stage group, the 21 patients were classified into either the response ($n = 8$) or nonresponse ($n = 13$) group based on the evaluation of treatment response (Fig. 1).

Table 1 summarizes the demographic and clinical characteristics of enrolled patients. Within the lesion groups, the advanced-stage HCC displayed significantly larger tumor size ($p < 0.0003$), greater tumor

number ($p = 0.0002$) and higher α -fetoprotein levels ($p = 0.002$) compared to the early-stage HCC (Table 2). Further comparison within the early-stage group ($\text{Ki-67} \leq 20\%$ versus $\text{Ki-67} > 20\%$) and the advanced-stage group (response versus nonresponse subgroup) shows no significant differences in the characteristics (Supplementary Tables 3 and 4).

Table 1
Demographic and clinical characteristics of patients with HCC

Characteristic	Patients with HCC
Patient	
Number	65
Age (years)	60.00 (51.50, 67.00)
Gender	
Male (Female)	56 (9)
Lesions Characteristic	
Tumor size (mm)	43.00 (24.00, 97.00)
Solitary tumor	43 (66.15)
Tumors 2–3 lesions	12 (18.46)
Tumors > 3 lesions	10 (15.38)
HBV Infection	58 (89.23)
Laboratory Information	
AFP (ng/mL)	26.09 (3.57, 369.97)
PAB (mg/L)	169.00 (118.00, 208.00)
ALB (mg/L)	40.00 (37.00, 93.00)
TP (g/L)	66.00 (63.00, 69.00)
ALT (U/L)	32.00 (19.00, 64.00)
AST (U/L)	44.00 (30.00, 77.00)
INR	1.06 (1.02, 1.16)
PT (s)	12.60 (12.00, 13.70)
PLT (×10 ⁹ /L)	142.00 (106.00, 186.00)
BCLC Stage	
Early-stage (BCLC 0-A)	40 (61.54)
Advanced-stage (BCLC B-C)	25 (38.46)
Data are presented as medians with IQR, or number (percentage). AFP, α-Fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; INR, international normalized ratio; PAB, prealbumin; PLT, platelets; PT, prothrombin time; SD, standard deviation; TP, total protein.	

Characteristic	Patients with HCC
Patient	
³¹ P 2D MRSI Characteristic	
Number of patients with both lesion and background data	16 (24.61)
Number of patients with lesion data	28 (43.08)
Number of patients with background data	21 (32.31)
Data are presented as medians with IQR, or number (percentage). AFP, α-Fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; INR, international normalized ratio; PAB, prealbumin; PLT, platelets; PT, prothrombin time; SD, standard deviation; TP, total protein.	

Table 2
Characteristics of patients with HCC in the lesion group

Characteristic	Early-Stage Group	Advanced-Stage Group	<i>p</i>
Number of patients	23	21	
Age (years)	64.00 (59.50, 67.50)	58.00 (49.00, 63.00)	0.028
Gender			
Male (female)	16 (7)	21 (0)	0.009
Lesions characteristic			
Tumor size (mm)	26.00 (20.50, 44.00)	103.00 (89.00, 175.00)	< 0.0003***
Solitary tumor	20 (86.96)	6 (28.57)	0.0002***
Tumors 2–3 lesions	3 (13.04)	8 (38.10)	
Tumors > 3 lesions	0 (0.00)	7 (33.33)	
BCLC stage	Stage 0-A	Stage B-C	
HBV infection	19 (82.60)	20 (95.24)	0.665
Laboratory information			
AFP (ng/mL)	5.43 (2.72, 216.57)	233.13 (57.08, 31101.00)	0.002**
PAB (mg/L)	160.39 ± 59.60	146.00 ± 59.31	0.427
ALB (mg/L)	41.00 (38.00, 96.00)	40.00 (35.00, 110.00)	0.518
TP (g/L)	63.83 ± 3.85	67.33 ± 6.10	0.026
ALT (U/L)	43.00 (16.50, 180.00)	33.00 (23.00, 40.00)	0.404
AST (U/L)	35.00 (23.00, 61.50)	63.00 (44.00, 123.00)	0.020
INR	1.06 (1.02, 1.15)	1.07 (1.04, 1.19)	0.317
Data are presented as medians with IQR, mean ± SD, or number (percentage). AFP, Alpha-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; INR, international normalized ratio; PAB, prealbumin; PLT, platelet; PT, prothrombin time; S, stage; SD, standard deviation; TP, total protein.			
* <i>p</i> < 0.017; ** <i>p</i> < 0.003; *** <i>p</i> < 0.0003 (Independent-samples t test, Mann–Whitney test, chi-square test, or Fisher’s exact test with a Bonferroni correction).			

Characteristic	Early-Stage Group	Advanced-Stage Group	<i>p</i>
PT (s)	12.60 (12.10, 13.70)	12.90 (12.20, 14.20)	0.431
PLT (×10 ⁹ /L)	137.00 (104.50, 164.50)	155.00 (135.00, 187.00)	0.093
Treatment	Resection	HAIC with or without conversion therapy	
³¹P 2D MRSI characteristic			
Number of patients with both lesion and background data	10 (52.17)	6 (28.57)	0.360
Number of patients with lesion data	13 (56.52)	15 (71.43)	
Data are presented as medians with IQR, mean ± SD, or number (percentage). AFP, Alpha-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; INR, international normalized ratio; PAB, prealbumin; PLT, platelet; PT, prothrombin time; S, stage; SD, standard deviation; TP, total protein.			
* <i>p</i> < 0.017; ** <i>p</i> < 0.003; *** <i>p</i> < 0.0003 (Independent-samples t test, Mann–Whitney test, chi-square test, or Fisher's exact test with a Bonferroni correction).			

Metabolic features of HCC compared to background liver tissue

Representative ³¹P 2D MRSI of the HCC and background voxel from a 77 years old man is displayed in Fig. 2. There was no significant difference in SNR between background and lesion voxels (56.46 ± 27.25 vs. 52.26 ± 29.60, *p* = 0.51).

HCC voxels are characterized by significantly higher mean ratios of PME/PDE (0.91 vs. 0.50, *p* < 0.0003), PE/PDE (0.50 vs. 0.28, *p* = 0.024), PME/TPC (0.18 vs. 0.14, *p* = 0.010), and PE/TPC (0.11 vs. 0.08, *p* < 0.0003), along with a lower PDE/TPC (0.22 vs. 0.28, *p* = 0.024) ratio (Fig. 3), when compared to the background liver. Similarly, the mean ratios of PME/(PME + PDE), PME/γ-ATP, PC/PDE, PE/TPC, PC/GPC, PC/Pi, PE/(PME + PDE), PE/GPC, PE/GPE PE/Pi and PE/γ-ATP were significantly elevated in the HCC voxels relative to the background voxels, as detailed in Supplementary Table 5.

Metabolic features in various tumor aggressiveness subgroups

To explore the relationship between metabolic indices and tumor aggressiveness, the patients were further stratified by BCLC stage, Ki-67 expression, and HAIC response. Based on the BCLC staging system, the metabolic features within the advanced-stage groups are characterized by significantly higher ratios of PME/PDE (1.41 vs. 0.71, *p* = 0.0005) and PE/PDE (0.73 vs. 0.40, *p* = 0.001) (Fig. 4a and 4b) compared to the early-stage group. Similarly, the mean ratios of PME/TPC, PME/(PME + PDE), PE/TPC,

PE/(PME + PDE), PE/GPC, and PE/GPE were significantly higher in the advanced-stage group compared to the early-stage group, as listed in Supplementary Table 6. Similar metabolic features were observed in the high Ki-67 lesions within the early-stage group, which exhibited significantly higher mean ratios of PME/PDE (0.73 vs. 0.51, $p = 0.010$), PE/PDE (0.44 vs. 0.27, $p = 0.004$) (Fig. 4c and 4d), PME/(PME + PDE), PE/TPC, and PE/(PME + PDE) (Supplementary Table 7) compared with low Ki-67 lesions.

However, within the advanced-stage group, the association between aggressiveness and metabolic features is relatively inverted. Nonresponsive lesions are characterized by a significantly lower mean ratio of PME/PDE (1.05 vs. 2.19, $p = 0.0001$), PE/PDE ratios (0.63 vs. 1.02, $p = 0.010$) (Fig. 4e and 4f), PME/TPC and PE/TPC (Supplementary Table 8) compared to the response subgroup, although these values are higher than their counterparts in the early-stage.

Discussion

Using ^{31}P 2D MRSI, this study characterized the metabolic profiles of HCC and revealed the association between altered cell membrane metabolism and tumor aggressiveness. Comparisons were made between HCC lesions and the surrounding liver, between early- and advanced-stage tumors, and within each stage. PME/PDE, PE/PDE ratios, along with other ratios where PME and PE (with PME comprised of PC and PE) are in the numerator, were significantly higher in all comparisons, except for the comparison within the advanced-stage group. The PME/PDE and PE/PDE ratios were significantly reduced in treatment-nonresponsive versus responsive HCC within the advanced-stage group. These findings suggest that ^{31}P 2D MRSI-derived metabolic indices may serve as potential indicators of tumor aggressiveness.

As mentioned earlier, previous studies have reported the elevated PME/PDE ratio in HCC compared to healthy liver tissues, though these studies were based on very small sample sizes [12, 13]. This study revealed significantly relatively elevated levels of PME, along with the decreased level of PDE in HCC compared to background tissue, based on 44 HCC lesions and 37 background voxels. These findings provide a characterization of the metabolic features of HCC, strongly confirming previous studies [12, 13] and may suggest enhanced membrane synthesis in HCC. Moreover, the present study achieved tumor localization by the ^{31}P 2D MRSI sequence, and the SNR did not differ significantly between lesion and background voxels, further supporting the validity and robustness of the HCC metabolic features relative to the background tissue.

In order to profile the relationship between the metabolic features and tumor aggressiveness, we initially compared early- and advanced-stage, and low and high Ki-67 expression of HCCs. PME/PDE ratio was significantly increased in advanced-stage versus early-stage lesions, and in high Ki-67 expression HCC lesions versus low Ki-67 expression lesions. These findings indicate an increased membrane synthesis and reduced membrane catabolism [31] in more aggressive HCC. In addition, Nwosu et al [6] found that most membrane lipid metabolism-related genes are upregulated and correlate with HCC

aggressiveness, as identified through online databases. Our study further extends their findings from online gene-datasets to detectable *in vivo* metabolic markers.

PE is one of upstream metabolites of PDE and regulated by a rate-limiting enzyme PE cytidyltransferase (PCYT2) in phospholipid biosynthesis [32]. Among PME components, PE exhibited the significantly increased PE/PDE ratio in HCC versus background, in advanced-stage lesions versus early-stage lesions, and in high Ki-67 expression HCC lesions versus low Ki-67 expression lesions. These findings suggest the accumulation of PE in HCC lesions are linked with increased aggressiveness. Previous studies have demonstrated that the reduced PCYT2 activity is associated with enhanced tumor aggressiveness [33, 34]. This reduction may disrupt the conversion of PE to its downstream phospholipids, leading to the accumulation of PE in more aggressive tumors. Our results proposed that ^{31}P 2D MRSI may allow for an indirect assessment of PCYT2 activity thereby to assess tumor aggressiveness in HCC.

However, subgroup analysis within the advanced-stage tumors revealed that nonresponding HCC lesions exhibited the comparatively lower PE/PDE and PME/PDE ratios versus treatment-responsive tumors, although the values are still higher than their counterparts in the early-stage group. These results suggest a greater depletion of cell membrane precursors in the nonresponse group compared to the response group within advanced-stage HCC. Chemotherapy resistance of HCC has been linked to the dysregulated glycosylation, which is driven by the elevated PCYT2 expression in advanced-stage tumor tissue [35, 36]. The upregulation of PCYT2 may accelerates the consumption of PE and related membrane components, which may account for the relatively lower PE/PDE and PME/PDE ratios observed in nonresponse lesions. These results reveals different metabolic patterns within the advanced-stage HCC and extends previous studies from *in vitro* evidences to the *in vivo* metabolic indices [35, 36]. Together, ^{31}P 2D MRSI could reflect tumor aggressiveness by quantifying the underlying cell membrane metabolic profile in HCC.

LIMITATIONS

Our study had several limitations. First, ^{31}P 2D MRSI utilized a coarse acquisition grid (4×4 voxels) with Hamming filtering, substantially limiting spatial resolution and causing significant partial volume effects. Employing a higher-resolution matrix with reduced voxel sizes would cause too low SNR with the currently used RF coil. Within the same scan time, the Hamming weighted averages could be exchanged with more spatial encodings, however at the cost of highly discretized Hamming weighting, which will reduce SNR. Second, adiabatic excitation generally requires a TR of about $1.25 \times T_1$, which should be around 8 s for optimal detection of PME and PDE. However, shortening the TR to 4 s, reduces the scan time by half and makes the protocol more suitable for clinical application, yet causes sub-optimal SNR and provides more T1 contrast. Third, the slice selection is based on an ISIS technique that assumes fully relaxed spectra (i.e. $\text{TR} > 5 \times T_1$) and absence of motion between the two ISIS scans to ensure excellent spatial selectivity. In our study, the compromised selectivity seems acceptable considering the relatively low signals of PCr from non-selected muscle that we observe. Fourth, the β -ATP signal was

excluded due to insufficient excitation bandwidth; pulse sequence optimization is needed for full ATP spectrum capture. Fifth, the used RF coil has a limited field of view with a steep gradient in the sensitivity profile. This provides a bias to detecting lesions only in close proximity to the coil. Moreover, the true voxel dimension is very large compared to the spatial sensitivity profile of the coil, which provides variance in the data when metabolite ratios or T1 values differ within the voxel.

Despite the limitations, we did find significant differences across various clinicopathologic subgroups. Therefore, our results may stimulate vendors in providing RF coil setups for the widely available 3T MRI systems that can excite uniform B1 fields and combined with local receiver arrays give maximal ^{31}P SNR over the entire liver, as shown for 7T [15], so to improve spatial resolution and spectral accuracy for translation to clinical applicability in the distinction of the aggressiveness of HCC.

CONCLUSION

This study has demonstrated that ^{31}P 2D MRSI could noninvasively detect metabolic features of HCC and the metabolic reprogramming can be linked to the HCC aggressiveness. Distinct alterations in phosphorus-containing metabolites were observed across various clinicopathologic subgroups classified by stage, proliferative activity, and chemotherapy response. Therefore, ^{31}P 2D MRSI has a great potential for *in vivo* assessment of HCC aggressiveness and prediction of tumor proliferative activity and chemotherapy response.

Abbreviations

ATP, Adenosine triphosphate;

BCLC, Barcelona clinic liver cancer;

GPC, glycerophosphocholine;

GPE, glycerophosphoethanolamine;

HAIC, hepatic arterial infusion chemotherapy;

HCC, hepatocellular carcinoma;

IQR, interquartile range;

PC, phosphocholine;

PCr, phosphocreatine;

PDE, phosphodiester;

PE, phosphoethanolamine;

Pi, inorganic phosphate;

PME, phosphomonoester;

^{31}P 2D MRSI, phosphorus-31 two-dimensional magnetic resonance spectroscopic imaging;

^{31}P MRSI, phosphorus-31 magnetic resonance spectroscopic imaging;

SD, standard deviation;

SNR, signal-to-noise ratio.

Declarations

Competing Interests

Peng Wu reports a relationship with Philips Healthcare, China that includes: employment. Jinyuan Weng reports a relationship with Philips Healthcare China that includes: employment. Other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

Jingzhe Li: Formal analysis, Writing - original draft. Yuchen Yang: Data curation, Resources, Project administration. Rong Deng: Methodology, Jiahao Zhou: Formal analysis, Validation. Xinxin Xu: Formal analysis, Validation. Huanhuan Chong: Funding acquisition, Peng Liu: Data curation, Peng Wu: Software, Supervision, Writing - review and editing. Jinyuan Weng: Software, Validation, Investigation. Yongjun Chen: Project administration. Rui Chang: Project administration. Haipeng Dong: Project administration. Ruokun Li: Project administration. Dennis W. J. Klomp: Writing - review and editing. Huimin Lin: Conceptualization, Writing - review and editing, Funding acquisition, Supervision. Fuhua Yan, Conceptualization, Writing - review and editing, Project administration. All authors reviewed the manuscript.

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Figures

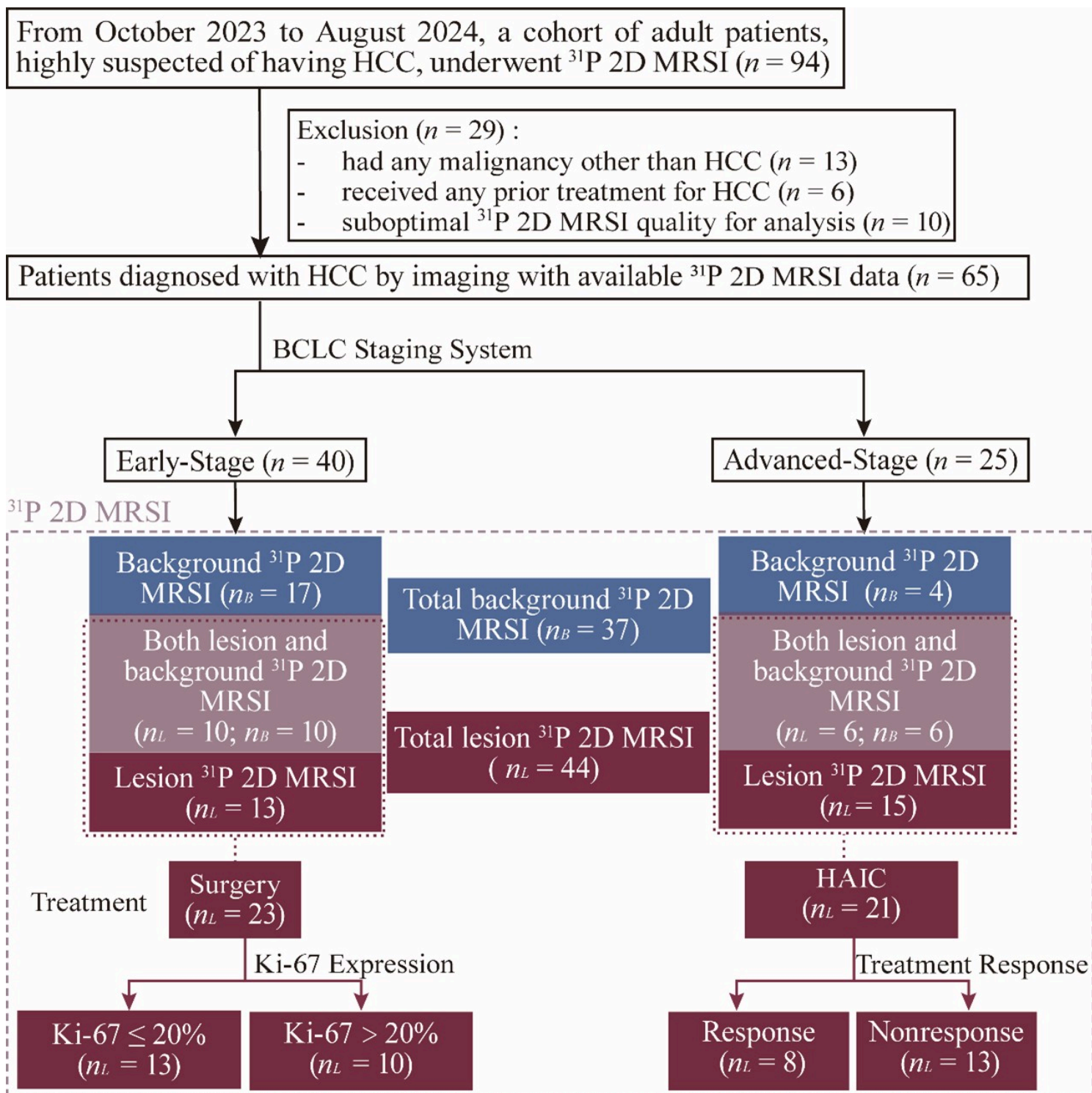


Figure 1

Flowchart of the study participants. HAIC, Hepatic arterial infusion chemotherapy; HCC, hepatocellular carcinoma; ^{31}P 2D MRSI, phosphorus-31 two-dimensional magnetic resonance spectroscopic imaging; n , number of patients; n_B , number of patients who provided ^{31}P 2D MRSI data from background voxels; n_L , number of patients who provided ^{31}P 2D MRSI data from lesion voxels

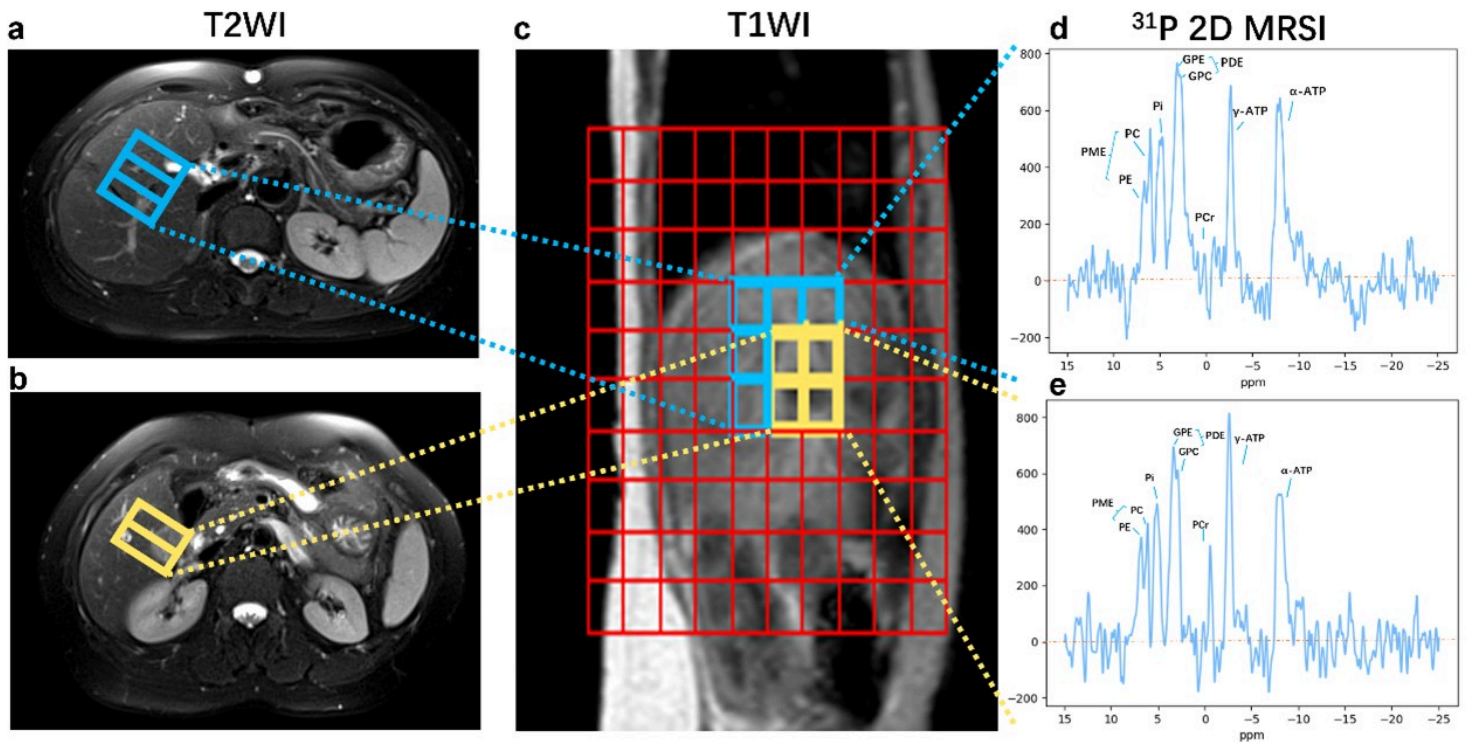


Figure 2

Representative ^{31}P 2D MRSI of an HCC lesion and the surrounding liver. Representative ^{31}P 2D MRSI from a 77-year-old male with HCC, with corresponding T2- and T1-weighted images for voxel localization. Liver background voxels are outlined in blue (a, c), and lesion voxels in yellow (b, c). Panels (d) and (e) show the corresponding ^{31}P spectra of the liver background and lesion, respectively. ATP, Adenosine triphosphate; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; HCC, hepatocellular carcinoma; PC, phosphocholine; PCr, phosphocreatine; PDE, phosphodiester; PE, phosphoethanolamine; Pi, inorganic phosphate; PME, phosphomonoester; ^{31}P 2D MRSI, phosphorus-31 two-dimensional magnetic resonance spectroscopic imaging; T1WI, T1-weighted imaging; T2WI, T2-weighted imaging

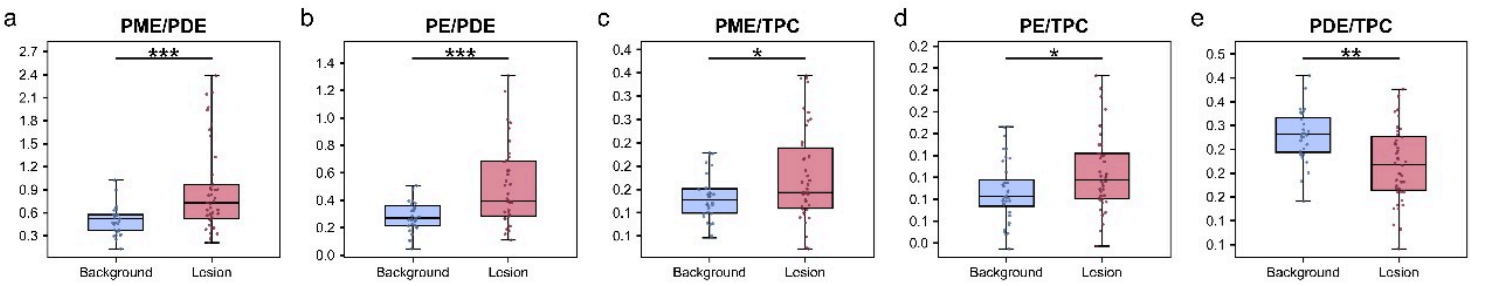


Figure 3

Metabolic features in HCC lesions compared to liver background. Elevated (a) PME/PDE, (b) PE/PDE, (c) PME/TPC, and (d) PE/TPC ratios, along with decreased (e) PDE/TPC ratios in HCC lesions compared to

background voxels. HCC, hepatocellular carcinoma; PDE, phosphodiester; PE, phosphoethanolamine; PME, phosphomonoester.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Independent-samples t test)

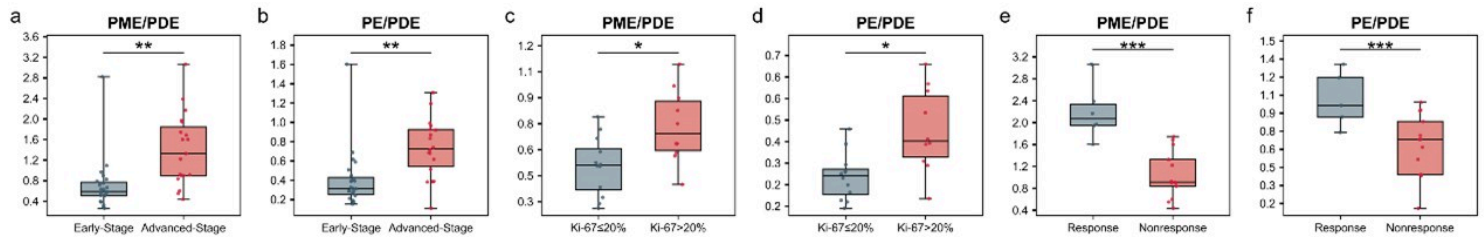


Figure 4

Elevated PME/PDE and PE/PDE ratios in HCC with increased tumor aggressiveness voxels. (a-b) Advanced- vs. early-stage groups, (c-d) high- vs. low-Ki-67 expression subgroups in early-stage HCC. (e) Decreased PME/PDE and PE/PDE ratios in nonresponse vs. response subgroups in advanced-stage HCC. HCC, hepatocellular carcinoma; PDE, phosphodiester; PE, phosphoethanolamine; PME, phosphomonoester

* $p < 0.0167$, ** $p < 0.0033$, *** $p < 0.0003$ (Independent-samples t test with a Bonferroni correction)

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