Low DMT1 expression associates with increased oxidative phosphorylation and early recurrence in HCC

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Abstract

Background—Despite a high rate of recurrences, long-term survival can be achieved after the resection of hepatocellular carcinoma (HCC) with effective local treatment. Discovery of adverse prognostic variables to identify patients with high risk of recurrence could improve the management of HCC. Accumulating evidence showing a link between carcinogenesis and increased expression of iron import proteins and intracellular iron prompted us to investigate a role

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of divalent metal-ion transporter-1 (DMT1) that binds and regulates a variety of divalent metals in HCC.

Materials and Methods—Clinical and gene expression data from RNA-seq in 369 HCC patients were obtained from The Cancer Genome Atlas (TCGA). Disease-free survival (DFS) was compared between *DMT1* high- and low-expressing tumors, and gene set enrichment analysis (GSEA) was conducted.

Results—Patients with lower expression of *DMT1* exhibited significantly worse DFS compared with the *DMT1* high group ($P = 0.044$), notably in advanced stage patients ($P = 0.008$). *DMT1* expression did not differ in etiologies, stages, and differentiation status of HCC. Interestingly, *DMT1* expression levels inversely associated with cellular respiratory function in HCC. Furthermore, GSEA revealed that metabolism-related gene sets such as glycolysis, oxidative phosphorylation, and reactive oxygen species pathway were significantly enriched in the *DMT1* low-expressing HCC.

Conclusions—Low *DMT1* expression associates with increased oxidative phosphorylation as well as glycolysis and identifies early recurrence in HCC patients after surgical treatment.

1. Introduction

Incidence of liver cancer has more than tripled since 1980. It has increased by about 3% per year in the U.S. from 2004 to 2013. An estimated 42,220 new cases of liver cancer will be diagnosed in the U.S. during 2018, and approximately three fourths of which will be hepatocellular carcinoma (HCC).[1] HCC is the sixth most frequently diagnosed cancer and responsible for the second most common cause of cancer mortality worldwide with estimated 700,000 deaths per year.[2,3]

HCC generally develops in patients with underlying chronic liver disease, which include cirrhosis from any cause such as chronic infection of hepatitis B virus (HBV) or hepatitis C virus (HCV), excessive alcohol intake, and nonalcoholic fatty liver disease (NAFLD). NAFLD and its advanced form, nonalcoholic steatohepatitis (NASH), which occurs with metabolic syndrome, obesity, and diabetes mellitus (DM), are now increasingly frequent underlying liver disease in patients with HCC.[4–8] Furthermore, numbers of clinical observational studies have provided a link between obesity and risk of HCC.[9–13] Several studies have indicated an association between the sequelae of NAFLD and the development of HCC even in the absence of cirrhosis;[8,14,15] however, pathogenesis of HCC development in NAFLD/NASH remains to be elucidated.

For patients who are medically fit with healthy background liver function and have resectable disease, liver resection continues to be a mainstay treatment.[16,17] However, even with complete surgical extirpation, recurrence rates within 5 years have been reported to exceed 70%.[18,19] Since the majority of recurrences are intrahepatic due to local recurrence

or a new second primary tumor,[17,20,21] the goal of post-resectional surveillance is early detection of disease that might be amenable to subsequent local therapy such as repeat liver resection, liver transplantation, thermal ablation, and transarterial chemoembolization (TACE) or radioembolization.[22–25]

Several tumor-related biologic as well as histologic factors such as high preoperative alpha fetoprotein (AFP) levels, tumor size, vascular invasion, resectional margin status, spontaneous tumor bleeding, and poor histologic grade of differentiation have been identified as potential predictors of recurrence,[17,20,21,24] yet the management of surveillance remains challenging and additional indicators will improve the detection of recurrence in HCC. Recent advances in RNA-seq transcriptomics allow identification of novel transcripts and molecular mechanism governing carcinogenesis, progression, and prognosis in solid malignancies.

Divalent metal-ion transporter-1 (DMT1) is a transmembrane protein involved in transportation of divalent metals including cadmium, cobalt, copper, nickel, lead, manganese, zinc, and in particular iron.[26,27] DMT1 is ubiquitously expressed, most notably in proximal duodenum, immature erythroid cells of the bone marrow, brain, and kidney.[28] Dietary iron is imported into the enterocyte through DMT1 on its apical surface and enters into the blood stream through ferroportin 1 (Fpn1).[29] Once absorbed, transferrin-bound iron is endocytosed and released into hepatocytes via DMT1.[30–32] Non-transferrin–bound iron, in case of iron overload, is also taken up by hepatocytes though DMT1 directly.[33] Some degrees of iron overload have been shown to be present in 10% to 30% of patients with chronic liver disease, and known as one of the causes of hepatocarcinogenesis through generating reactive oxygen species.[34] We previously reported aberrant expression of *DMT1* in the duodenum, where iron absorption takes place, led to liver iron accumulation in patients with NASH.[35] Boult *et al.* investigated the expression of iron transport proteins in the premalignant lesions, Barrett's metaplasia and esophageal adenocarcinoma, and found that progression to adenocarcinoma was associated with increased expression of iron transport proteins including DMT1.[36] Moreover, Brookes *et al.* showed progression of normal colon and precancerous state such as low/high grade dysplastic adenomas to colorectal carcinoma was associated with increased expression of DMT1.[37]

While DMT1 is involved in the carcinogenesis, its contribution to tumor progression and relapse of HCC remains unclear. Interestingly, *DMT1* expression was found to be up-regulated in iron-loaded, non-cirrhotic, and non-tumorous liver tissues compared with normal liver controls.[38] However, Deugnier *et al.* described lack of iron accumulation within HCC in hereditary hemochromatosis patients,[39] indicating the role of DMT1 differs between hepatocarcinogenesis and tumor progression. To date, no study has been reported on the significance of *DMT1* gene expression in the context of recurrence following liver resection. In the present study, we employed publicly available database, The Cancer Genome Atlas (TCGA) for genomic analysis to elucidate a role of DMT1 in recurrence after liver resection for HCC and performed gene set enrichment analysis (GSEA) to interpret genome-side expression profile.

2. Materials and Methods

Data acquisition and pre-processing

The National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI) obtained human data from doctors who collected tissue for TCGA after gaining approval with informed consent documents and local Institutional Review Boards (IRBs). The TCGA Biospecimen Core Resource laboratory extracted RNA from all samples and distributed the RNA to their Genome Sequencing Centers, where the uniform sequencing technique was used by TCGA researchers. There were 440 HCC cases of liver resection in TCGA cohort (http://cancergenome.nih.gov). RNA seq v2 z-scores and clinical data were obtained through the cBioportal for Cancer Genomics (http://cbioportal.org) on January 15th, 2018, and processed as previously described.[40,41] Of 440 cases, there were 369 primary tumors available for gene expression data from RNA-sequence. Two recurrent tumor samples from two patients whose primary tumors were also registered were excluded from our study to avoid duplication of patients. We also excluded two patients with neoadjuvant therapy to eliminate the gene expression affected by therapy prior to surgery. Patients were divided into *DMT1* high and *DMT1* low groups according to their gene expression levels with a cutoff being determined as the lower quartile value.

GSEA

GSEA was performed comparing *DMT1* high and low patients utilizing the Java GSEA implementation version 3.0 provided by the Broad Institute (http://software.broadinstitute.org/gsea/index.jsp) as described previously.[42–45]

Statistical Analysis

Statistical analyses were performed using unpaired two-sided Student's *t* test or one-way ANOVA followed by Tukey's test for continuous variables when appropriate and Fisher's exact test for categorical variables. Disease-free survival (DFS) was estimated by the Kaplan–Meier (KM) method and the log-rank test. Censoring times were designated with vertical tick-marks on the KM curves. Correlations between *DMT1* and the other gene mRNA expression levels were assessed with Pearson's correlation coefficients. *P* values of less than 0.05 were considered statistically significant. Data analyses and creation of graphs were carried out using R version 3.4.3 (http://www.r-project.org) and Bioconductor packages (http://www.bioconductor.org).

3. Results

TCGA liver hepatocellular carcinoma patient cohort

Of 440 HCC cases in the whole TCGA hepatocellular carcinoma cohort, gene expression data from RNA-seq of primary tumor without neoadjuvant therapy were available for 369 patients. The mean age of the cohort was 59.4 ± 27.0 years-old. The prevalence of HBV, HCV, dual HBV-HCV, and non-B non-C were 96 (26.0%), 48 (13.0%), 7 (1.9%), and 198 (53.7%), respectively (Fig. 1A). There were also 7 (1.9%) hemochromatosis patients. Of the 369 patients who underwent liver resection, the numbers of single segmentectomy, multiple segmentectomy, lobectomy, extended lobectomy, and liver transplantation were 88 (23.8%),

86 (23.3%), 140 (37.9%), 25 (6.8%), and 1 (0.3%), respectively (Fig. 1B). The proportions of patients with AJCC stage I, II, III, and IV were 169 (45.8%), 85 (23.0%), 85 (23.0%), and 5 (1.4%), respectively (Fig. 1C). The median observation period was 19.3 months (range, 0–120.7m). The estimated 3- and 5-year DFS rates were 38.1% and 28.7%, and the 3- and 5-year OS rates were 62.5% and 47.8%.

Decreased *DMT1* expression is associated with worse DFS in HCC patients after liver resection

Next, 369 patients were divided into *DMT1* high and *DMT1* low groups. DFS data were available in 317 HCC patients who underwent surgical resection, and there were 242 *DMT1* high and 75 *DMT1* low patients. We found the *DMT1* low group showed significantly worse DFS compared with the *DMT1* high group ($P = 0.044$) (Fig. 2A). To explore potential correlation between DFS and *DMT1* expression in different stages, subgroup analyses were conducted in early-stage (AJCC stage I) patients and advanced-stage (AJCC stage II, III, and IV) patients. The DFS of *DMT1* high and low groups were similar in 148 early-stage patients ($P = 0.815$), whereas the *DMT1* low group showed significant worse DFS compared with the *DMT1* high group in 148 advanced-stage patients ($P = 0.008$) (Fig. 2B and C). High expression of DMT1 has been reported to contribute to carcinogenesis, while our data illustrated low expression of DMT1 caused worse prognosis, suggesting the role of DMT1 might be different between carcinogenesis and tumor progression.

DMT1 expression does not differ in etiologies, stages, and differentiation status of HCC

To identify the features of the low *DMT1*-expressing HCC associated with worse DFS, we evaluated the expression of *DMT1* in etiologies, stages, and differentiation status of HCC. We found that *DMT1* expression levels were independent of virus infectious status (Fig. 3A), and comparable in different stages (Fig. 3B). The comparison of clinicopathological characteristics between *DMT1* high (n = 277) and low (n = 92) groups were shown in Table 1. There was no difference in the any features listed between *DMT1* high and low groups. There was also no difference in *DMT1* expression levels among grade 1 (well differentiated), grade 2 (moderately differentiated), and grade 3/4 (poorly differentiated/ undifferentiated) (Fig. 3C). As NAFLD is a growing cause of HCC and a manifestation of metabolic syndrome, we sought to investigate the relation between *DMT1* expression and metabolic status. There was little information available on metabolic syndrome including diabetes in this cohort. We compared 66 patients with BMI > or = 30, which indicates high risk for diabetes, with 265 patients with BMI < 30, and there was no difference of *DMT1* expression (Fig. 3D). Taken together, we found *DMT1* expression levels were not associated with underlying disease, or any known factors related to prognosis or recurrence of HCC. Of note, *K-ras* and *H-ras* are known as oncogenes found in less than 7% of human liver cancers, [46] and the activation of Ras pathway has a role in HCC initiation and progression.[47] Loss of TP53 function, which is a frequently mutated tumor suppressor gene in HCC, has been shown to be associated with hepatocellular carcinogenesis[48] and poor prognosis.[49] Therefore, we looked at P53 pathway, K-ras, and H-ras signaling in the GSEA; however, we did not find any significant enrichments of those gene sets (Fig. 3E).

DMT1 expression is not associated with iron regulatory or heme transport genes in HCC

Since DMT1 is known as an iron importer, we evaluated correlation between *DMT1* expression and iron regulatory genes or heme transport gene expressions in HCC. These include ceruloplasmin, cytochrome b reductase (Dcytb), Fpn1, hepcidin, hephaestin, iron-responsive element-binding protein 1 (IRP1), iron-responsive element-binding protein 1 (IRP2), transferrin receptor 1 (TfR1), transferrin receptor 2 (TfR2), transferrin (Trf), and solute carrier family 39 member 14 (Zip14) as iron regulatory genes, and hemoglobin scavenger receptor (CD163) and low density lipoprotein receptor-related protein 1 (LRP1/CD91) as heme transport genes. Despite its function known to be as an iron importer, *DMT1* expression correlated with neither of the iron regulatory genes nor heme transport genes (Fig. 4A and B). Furthermore, GSEA revealed that none of iron regulatory or heme transport-related gene sets were enriched in *DMT1* high group (Fig. 4C).

DMT1 expression level inversely associates with oxidative phosphorylation and glycolysis in HCC

In order to identify the features of the low *DMT1*-expressing HCC, which have worse DFS after liver resection, GSEA was conducted based on the 50 hallmark gene sets.[44] Intriguingly, oxidative phosphorylation and glycolysis gene sets were found to be enriched in *DMT1* low tumors in addition to DNA repair and reactive oxygen species gene sets (Table 2). Respiratory metabolism-related gene sets, such as glycolysis (NES = −1.547, *P* = 0.022), oxidative phosphorylation (NES = −2.166, *P* < 0.001), which generates adenosine triphosphate (ATP) using pyruvate, a product of glycolysis, and reactive oxygen species pathway (NES = −1.766, *P* = 0.011), were enriched in the low *DMT1*-expressing HCC among 50 hallmark gene sets (Fig. 5A and B). Furthermore, respiratory chain known to be a major source of reactive oxygen species and the site of oxidative phosphorylation was also enriched in *DMT1* low group (NES = −2.148, *P* < 0.001) (Fig. 5B). Our unexpected findings of inverse correlation between *DMT1* and oxidative phosphorylation and glycolysis in HCC patients prompted us to further examine mitochondrial metabolism-related gene sets by GSEA. Since oxidative phosphorylation is a process that takes place in mitochondria, as we expected, "Mitochondrion pathway", a broad function of mitochondria, was found to be significantly enriched in *DMT1* low tumor (NES = −2.099, *P* < 0.001) (Fig. 5C). Next, we further evaluated roles of DMT1 in regulation of other mitochondrial functions. Despite increased oxidative phosphorylation and mitochondrion pathway in *DMT1* low group, other known mitochondrial functions including citric acid cycle (Kreb pathway; NES = −1.302, *P* = 0.193), fatty acid beta-oxidation (mitochondrial fatty acid beta oxidation; NES = −1.199, *P* = 0.332), storage of calcium ions (calcium ion homeostasis; NES = −0.990, *P* = 0.428), or apoptosis (apoptotic mitochondrial changes; NES = −1.381, *P* = 0.053) were not enriched (Fig. 5D). These findings suggest that *DMT1* low tumors with higher risk of recurrence have increased glycolysis and oxidative phosphorylation facilitating higher ATP production.

Although glycolysis is known to be induced by HIF-1α in hypoxia,[50] hypoxia was not enriched in GSEA (NES = 0.688, *P* = 0.856) (Fig. 5E). HIF-1α and its upstream regulator, prolyl hydroxylase domain-containing protein 1 (PHD1) did not show strong correlations with *DMT1* expression (Fig. 5F). While protein secretion gene set was significantly enriched in the *DMT1* high group (Normalized enrichment score (NES) = 1.782, *P* = 0.008) (Table 2),

expression of *ALB*, which encodes the major protein exclusively synthesized in the liver, was not associated with *DMT1* expressions ($r = -0.125$, $P = 0.016$) (Fig. 5G).

4. Discussion

Despite a higher recurrence rate, long-term survival can be achieved after resection of HCC because in most cases recurrences are confined to the liver and may be amenable to local therapies.[17,20,21] Thus identification of risk factors, close follow-up evaluation, and early detection are of great importance.

DMT1 was first identified as a transmembrane iron-transport protein, and found to play an important role in intestinal iron absorption.[51] DMT1 is also expressed in the liver; however, a role of DMT1 in hepatocytes remains unclear. Although transferrin-bound iron as well as free iron is uptaken by hepatocyte via DMT1, Wang *et al*. showed that DMT1 in hepatocytes is dispensable for hepatic iron accumulation and non-transferrin-bound iron uptake using mice with the *Dmt1* gene selectively inactivated in hepatocytes (*Dmt1^{liv/liv}*).[52] In line with this, expression of *DMT1* did not correlate with iron regulatory genes or heme transport genes.

While iron deposition in hepatocyte has been shown to induce hepatocarcinogenesis, how iron and its importer, DMT1, are associated with cancer recurrence remains to be elucidated. In this study, we employed gene expression data from TCGA HCC cohort, and found that higher expression of *DMT1* in HCC was associated with significantly longer DFS after resection (Fig. 2A). This was somewhat an unexpected finding given the previous studies suggesting potential role of DMT1 in iron overload frequently seen in chronic liver disease and one of causes of hepatocarcinogenesis,[33,35] but might suggest potentially unrecognized roles of DMT1 in HCC.

In 1950s, Otto Warburg observed that cancer cells preferentially relied on aerobic glycolysis, which is less efficient than mitochondrial oxidative phosphorylation in terms of ATP generation, even in the presence of abundant oxygen.[53,54] For decades, the respiratory alteration had been regarded as a result of compensation for mitochondrial dysfunction, while recent studies have revealed oncogenes, such as *K-ras* and *c-Myc*, and hypoxia environment induce glycolysis.[50,55] Of note, iron is required as a cofactor for PHD1 to hydroxylate proline residues on HIF-α in regulation of hypoxia;[56] however, hypoxia pathway was not associated with *DMT1* expression levels in HCC in hallmark gene set (Fig. 5E), and we did not find significant correlation between *DMT1* expression and PHD1 (Fig. 5F) in the present study. Low *DMT1* group with worse DFS showed enrichments of both glycolysis and oxidative phosphorylation, suggesting ATP production depends on both glycolysis and oxidative phosphorylation. Although it is well known that glycolysis is enhanced in most malignant tumors, contribution of oxidative phosphorylation for ATP production to HCC growth remains elusive.[57] In this study, we found that HCC with low *DMT1* correlates not only with increased glycolysis but also with enhanced oxidative phosphorylation. While it is unclear whether oxidative phosphorylation is augmented in the process of an adaptation of hepatocellular carcinoma cells to meet energy demands in the tumor microenvironment, HCC usually develops blood supply predominantly from the

arterial system during progression,[58] and can provide more oxygen for oxidative phosphorylation than portal vein.

Some tumors are relatively glycolytic, the others have high rates of mitochondrial respiration.[57] Inactivation of the mitochondrial transcription factor has been shown to impaire lung tumor cell proliferation,[59] suggesting mitochondrial function is inevitable for cancer survival. Recently, Tan *et al.* have shown that tumor microenvironment instructs cancer cells to restore respiratory function, recover mitochondrial function, and reestablish tumor-initiating efficacy. [60] ATP is known as the main energy fuel and also reported to promote cell proliferation and drug resistance in cancer cells.[61] Since we have observed inverse association between *DMT1* and oxidative phosphorylation, our results might indicate that DMT1 plays a role in directly or indirectly inhibiting oxidative phosphorylation, or vice versa. Indeed, our findings of strong correlation between increased mitochondrial metabolism and worse prognosis are in line with the profound influence of mitochondrial metabolism on all steps of oncogenesis such as malignant transformation, tumor progression and response to treatment.[62,63]

Given cancer cells require higher levels of cytosolic ATP than normal tissue to sustain elevated rates of growth and division,[64] we assume elevated ATP is associated with worse prognosis in HCC. ATP is used for acquiring drug resistance in cancer cells through ATP-dependent efflux pump.[65] ATP also increases metastatic efficiency by improving ability of cancer cells to withstand traumatic deformation in the microvasculature of target organs.[66] Cancer cells secrete ATP into their microenvironment at high concentrations than healthy tissues.[67,68] The extracellular ATP is known to be unstable and can be hydrolyzed to ADP, AMP, and eventually adenosine, and the final degradation product suppresses cellular immunity in the tumor environment and stroma.[69]

DMT1 has thus far not been reported to be associated with activation of mitochondrial metabolism. Recently, DMT1 was found to be located in outer mitochondrial membrane and play roles in mitochondrial iron import and other metals. [70] The fact that iron is of vital importance for mitochondrial energy metabolism in oxidative phosphorylation as electron carriers contradicts our data where low *DMT1* expression associates gene enrichment of mitochondrial function. Since our study investigated oxidative phosphorylation between high and low *DMT1* expression and did not compared with *DMT1* expression in normal liver, the relatively low *DMT* expression does not necessarily reflect a shortage of DMT1 and iron. The involvement of other metals may underlie the association between *DMT1* low and up-regulated mitochondrial respiration.

The present study provides novel links between low DMT1 and increased mitochondrial and respiratory function in HCC with worse DFS, yet there are some limitations. First, this study was conducted using only one publicly available data without being validated using other cohorts. Second, this study is based on the gene expression of the primary tumor in TCGA cohort, that is, we have not verified through any *in vitro* or *in vivo* studies. To determine the role of *DMT1* gene in association with oxidative phosphorylation and glycolysis, further studies would be warranted.

5. Conclusions

Lower *DMT1* mRNA expression associates with poor DFS in patients with HCC regardless of underlying disease, stages, and differentiation status, and is associated with increased mitochondrial oxidative phosphorylation and glycolysis. Further studies are warranted to uncover how DMT1 affects the respiratory chain in mitochondria.

Acknowledgments

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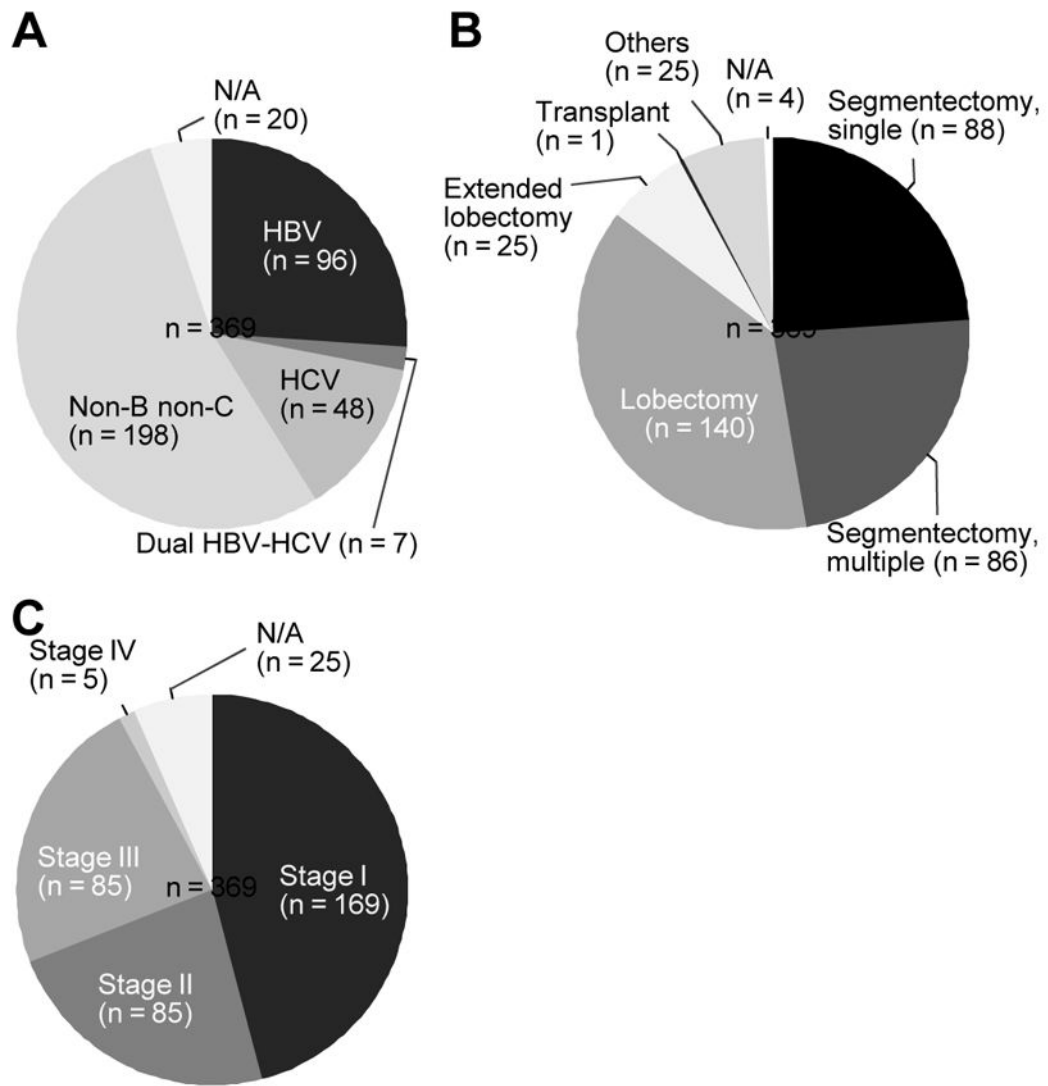
A

B

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Fig. 1. The distribution of etiologies, surgical procedures, and stages

(A) Pie chart showing the distribution of etiologies in 369 patients with HCC from The Cancer Genome Atlas (TCGA) Liver Hepatocellular Carcinoma (LIHC) dataset. (B) Pie chart showing the types of surgical procedures performed. (C) Pie chart showing AJCC stages.
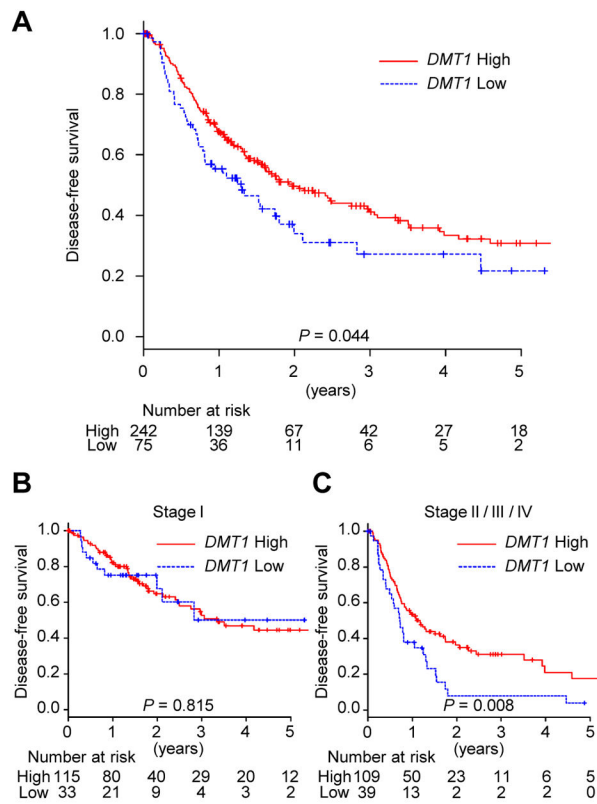
Fig. 2. Decreased *DMT1* mRNA expression indicates poor disease-free survival (DFS) in patients with HCC

(A) Kaplan-Meier (KM) estimates of DFS in whole patients (n=317) from TCGA. (B) KM estimates of DFS in patients with AJCC stage I (n=148). (C) KM estimates of DFS in patients with stage II/III/IV (n=148).
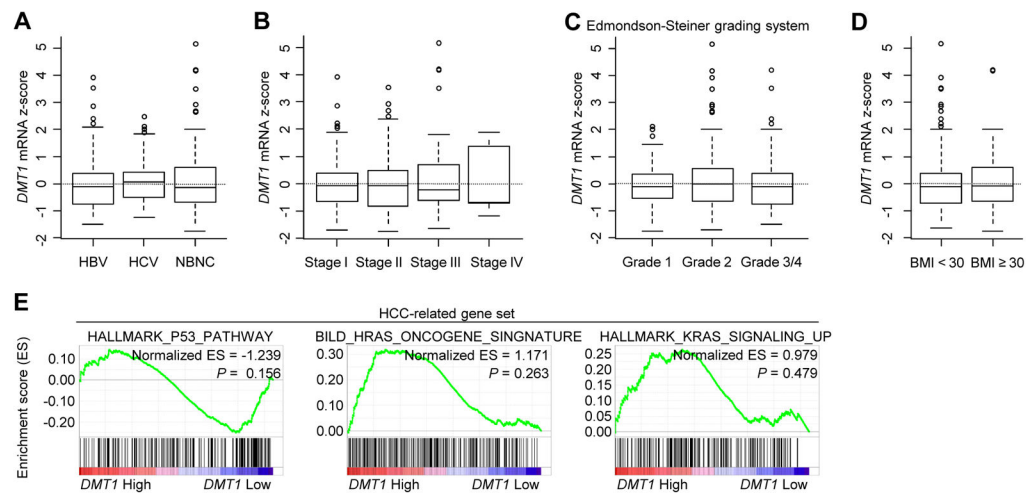
Fig. 3. *DMT1* expression does not differ in etiologies, stages, and differentiation status of HCC
(A) Box-plot diagram showing the *DMT1* mRNA expression levels in 96 patients with HBV, 48 patients with HCV, and 198 patients without HBV or HCV (NBNC) from TCGA. Seven patients with dual HBV and HCV were excluded from this analysis. (B) Box-plot diagram showing the *DMT1* mRNA expression levels of 169 patients with stage I, 85 patients with stage II, 85 patients with stage III, and 5 patients with stage IV. (C) Box-plot diagram showing the *DMT1* mRNA expression levels in 55 patients with Edmondson-Steiner grade 1, 175 patients with grade 2, and 133 patients with grade 3/4. (D) Box-plot diagram showing the *DMT1* mRNA expression levels of 66 patients with BMI > or = 30 and 265 patients with BMI < 30. (E) Enrichment plots of HCC-related gene sets comparing *DMT1* high and low.
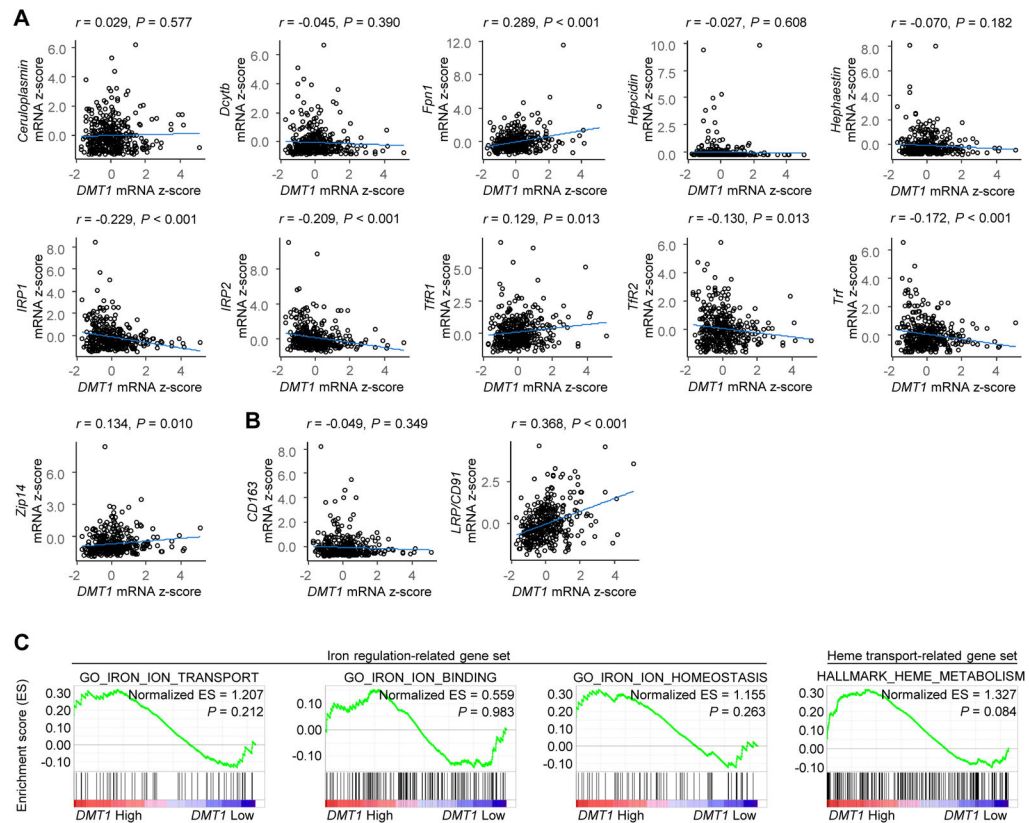
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Fig. 4. *DMT1* **mRNA expression correlates with no other iron regulatory genes or heme transport genes**

(A) Scatter plots between *DMT1* and iron regulatory gene mRNA expressions in HCC patients from TCGA. (B) Scatter plots between *DMT1* and heme transport gene mRNA expressions in HCC patients. (C) Enrichment plots of iron metabolism/heme metabolism-related gene sets comparing *DMT1* high and low.
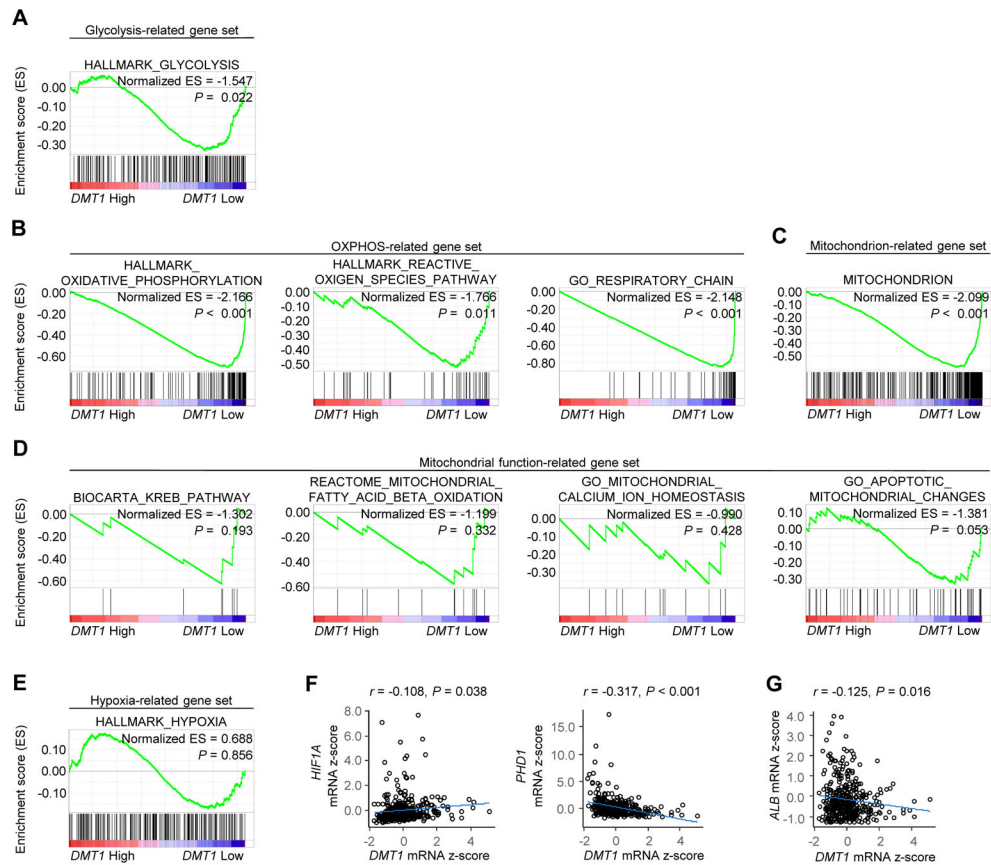
Fig. 5. Low *DMT1*-expressing HCC is associated with oxidative phosphorylation as well as glycolysis

(A) Enrichment plot of glycolysis gene set, (B) oxidative phosphorylation-related and reactive oxygen species gene sets, (C) mitochondrion gene set, (D) other mitochondrial function-related gene sets, (E) and hypoxia-related gene set comparing *DMT1* high and low HCC tumors from TCGA. (F) Scatter plots between *DMT1* and hypoxia-related gene mRNA expressions. (G) Scatter plots between *DMT1* and *ALB* gene mRNA expressions in HCC patients.

Table 1

Clinicopathological characteristics of patients from The Cancer Genome Atlas Liver Hepatocellular Carcinoma

	DMT1 High (n = 277)	*DMT1* Low (n = 92)	*P* value
Age, years, mean (range)	60(16–90)	59(17–81)	0.565
Gender (%)			
Male	189(68)	59(64)	0.444
Female	87(31)	33(36)	
Race (%)			
White	143(52)	39(42)	0.278
Black	14(5)	3(3)	
Asian	112(40)	45(49)	
BMI, kg/m^2, mean (range)	26(15–62)	25(17–38)	0.103
Child-Pugh (%)			
A	156(56)	58(63)	1.000
B/C	16(6)	5(5)	
Etiology (%)			
HBV	70(25)	26(28)	0.515
HCV	39(14)	9(10)	
NBNC	160(58)	57(62)	
Serum albumin, g/dL, mean (range)	3.9(1.2–8.0)	3.9(2.0–5.3)	0.946
AFP, ng/mL, median	15	11	0.066
Pathology stage (AJCC 6th) (%)			
Stage I	128(46)	41(45)	0.711
Stage II/III/IV	129(47)	46(50)	
Pathology T stage (%)			
T1/2	203(73)	69(75)	1.000
T3/4	70(25)	23(25)	
Vascular invasion (%)			
No	158(57)	47(51)	0.335
Yes	77(28)	30(33)	
Residual tumor (%)			
R0	240(87)	82(89)	0.397
R1/2	11(4)	6(7)	
Ishak fibrosis score (%)			
0	47(17)	27(29)	0.061
1/2	24(9)	7(8)	
3/4	23(8)	5(5)	
5/6	62(22)	14(15)	
Edmondson-Steiner grade (%)			
G1/2	177(64)	54(58)	0.259
G3/4	95(34)	38(41)	

Table 2

Summary of gene set enrichment analysis (GSEA) in hallmark gene sets

Gene sets enriched in *DMT1* high	Size	ES	NES	p-value
Protein secretion	95	0.474	1.782	0.008
Androgen response	96	0.391	1.520	0.039
Oxidative phosphorylation	195	−0.709	−2.166	< 0.001
DNA repair	142	−0.472	−1.828	0.006
Reactive oxygen species pathway	46	−0.525	−1.766	0.011
Glycolysis	195	−0.324	−1.547	0.022
UV response up	153	−0.282	−1.376	0.030

ES = enrichment score; NES = normalized ES