Downregulated expression of PBRM1 in sarcomatoid hepatocellular carcinoma

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Abstract. Sarcomatoid hepatocellular carcinoma (SHCC) is a rare and highly lethal subtype of HCC. The present study aimed to explore the unique markers of SHCC using whole gene expression analysis. Subsequently, gene expression analysis was performed using five sarcomatoid and seven carcinomatoid components of seven tissues from patients with SHCC. The results demonstrated a significant down-regulation of polybromo 1 (*PBRM1)* gene expression in the sarcomatoid components. Immunohistochemical staining also indicated a decreased expression of PBRM1 in the sarcomatoid components. Moreover, gene ontology enrichment analysis revealed that most of the 336 differentially expressed genes between the sarcomatoid and carcinomatoid components were involved in functions associated with DNA replication and histone methylation, which was consistent with the loss of function of PBRM1 which encodes Switch/sucrose-non-fermentable chromatin remodeling complex protein. Therefore, the results of the present study suggested that PBRM1 may be a candidate biomarker for the evaluation of SHCC.

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer and fourth leading cause of cancer-related death worldwide (1). HCC often presents as a hyperenhancement in the arterial phase of dynamic computed tomography (CT) or dynamic magnetic resonance imaging (MRI) and washout in the portal vein phase or equilibrium phase. The latter test has a sensitivity of 66-82% and a specificity of 91-92% (2). HCC cases with typical patterns can be diagnosed by contrast-enhanced ultrasound, CT or MRI, and pathological diagnosis is not required, which avoids the risk of tumor seeding via biopsy. Sarcomatoid HCC (SHCC) is a rare and highly lethal subtype of HCC that is characterized by the presence of spindle-shaped, pleomorphic or bizarre giant cells (3,4), with an incidence rate of 1.7-1.9% in post-operative HCC cases (5). It has been reported that SHCC is associated with a 3-year overall survival rate of <20% (6-8), and in some cases, repeated non-surgical therapies of HCC can lead to necrosis and degeneration of hepatocytes, resulting in SHCC (9). Compared with conventional HCC, SHCC has been reported to have larger tumor sizes, a higher incidence of lymph node metastasis, a higher proportion of advanced lesions and a significantly poorer overall and disease-free survival (5). Thus, SHCC is characterized by an aggressive clinical course and a high incidence of early recurrence (10).

At present, no standardized therapy has been established for this rare type of cancer, and its pathogenesis remains largely unclear. Previous integrated genomic analyses of HCC samples demonstrated that catenin b1 (40%) and *TP53* (21%) mutations are mutually exclusive and define two notable groups of HCC characterized by distinct phenotypes, but few studies have investigated the molecular features of SHCC (11). Sarcomatoid carcinoma of various tissues, including SHCC, is characterized pathologically by the presence of both carcinomatoid and sarcomatoid components, with intratumor heterogeneity and a propensity for intratumor transformation

from carcinomatoid to sarcomatoid type (12,13). The distinction between sarcomatoid and carcinomatoid components in SHCC is clinically important because of potential impact on treatment strategies and patient outcomes. Understanding the molecular characteristics of these components through whole transcriptome analysis may identify potential biomarkers for more accurate SHCC diagnosis, leading to improved patient care and tailored therapeutic interventions. Therefore, the aim of the present study was to clarify the molecular characteristics of the sarcomatoid component compared with the carcinomatoid component, based on the whole transcriptome, and to identify a new biomarker for the diagnosis of SHCC.

Materials and methods

Patients and tissue samples. A search for patients diagnosed with SHCC at three institutions [Kansai Medical University Hospital (Hirakata, Japan), Osaka Metropolitan University Hospital (Osaka, Japan) and Kindai University Hospital (Osakasayama, Japan)] over ~25 years (dating back from 2019) was conducted at the time of ethical approval. Subsequently, patients diagnosed with SHCC at the three institutions between 2006 and 2015 were retrospectively selected based on their medical records. This was due to specimens collected before 2006 not being preserved at the hospitals or being too old for use. In addition, no SHCC cases were identified between 2016 and 2019. From this pool, formalin-fixed, paraffin-embedded (FFPE) tumor tissues that had been collected and stored for diagnostic purposes at the time of surgery were obtained from 7 patients with SHCC who underwent surgical resection at Kansai Medical University Hospital (n=4), Osaka Metropolitan University Hospital (n=1) and Kindai University Hospital (n=2) between March, 2006 and December, 2015. The inclusion criteria for patient selection in the present study were as follows: Patients with HCC who underwent surgical resection and had carcinomatoid and sarcomatoid tumor components as confirmed by pathological diagnosis following tumor resection. No specific exclusion criteria were applied in the present study, except for the absence of an adequate amount of FFPE specimen for analysis. All patients provided written informed consent to participate in the present study and for tumor tissue sample collection for analysis. SHCC was diagnosed during the postoperative pathological examination by pathologists at each institution. SHCC specimens were further analyzed by the same pathologist during the present study. The present study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan (14). The Institutional Ethics Review Boards of Kansai Medical University Hospital (approval no. 2019074), Osaka Metropolitan University Hospital (approval no. 2020-185) and Kindai University Faculty of Medicine (approval no. 31-201) approved the present study.

Whole transcriptome analysis. The FFPE samples were subjected to RNA extraction using the Allprep DNA/RNA FFPE Kit (cat. no. 80234; Qiagen, Inc.) according to the manufacturer's instructions. The quality and quantity of the RNA were determined with NanoDrop 2000 device (Thermo Fisher Scientific, Waltham, MA) and RiboGreen dsDNA Assay Kit (cat. no. R11490; Thermo Fisher Scientific). The AmpliSeq Transcriptome Human Gene Expression Kit (cat. no. A26325; Thermo Fisher Scientific, Inc.) was subsequently used for whole transcriptome analysis according to the manufacturer's instructions. For library preparation, barcoded cDNA libraries were generated from 10 ng total RNA using the SuperScript VILO cDNA Synthesis Kit (cat. no. 11754050; Thermo Fisher Scientific, Inc.). The cDNA was then amplified for 16 cycles following addition of the PCR master mix and AmpliSeq human transcriptome gene expression primer pool (Thermo Fisher Scientific, Inc.). After multiplex PCR, IonXpress Barcode Adapters (cat. no. 4474517; Thermo Fisher Scientific, Inc.) were ligated to the PCR products and purified using Agencourt AMPure XP beads (cat. no. A63881; Beckman Coulter, Inc.). The purified libraries were quantified using the Ion Library TaqMan Quantitation Kit (cat. no. 4468802; Thermo Fisher Scientific, Inc.) and the adjusted to 50 pM with low TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA), and then pooled and sequenced using an Ion Torrent S5 system (Thermo Fisher Scientific, Inc.) and an Ion 550 Chip Kit (cat. no. A34538; Thermo Fisher Scientific, Inc.). The Ion Torrent S5 system produces single-end short-reads with 120-bp read length. Base calling, alignment to the human reference genome (hg19) and quality control were performed using Ion Torrent Suite v5.12 software (Thermo Fisher Scientific, Inc.). The raw reads were analyzed using the AmpliSeqRNA plugin to generate gene-level expression values for all 20,802 human reference sequence (Seq) genes.

Gene selection and pathway analysis. Data were processed with Transcriptome Analysis Console (ver. 4.0.3; Thermo Fisher Scientific, Inc.) to determine the differentially expressed genes (DEGs). DEGs were selected using |fold change| >2 and $P<0.05$ as the cut-off. Hierarchical clustering of these genes was performed using the mean linkage method with 1-Pearson correlation coefficient as the distance measure using Morpheus (software.broadinstitute.org/morpheus/). Functional and pathway enrichment analysis was performed using Metascape (ver. 3.5; accessed on 21 Feb 2023) (15).

Immunohistochemistry (IHC). FFPE sections of 4-μm thickness were stained for polybromo 1 (PBRM1) using a validated and published IHC method (16,17). Briefly, FFPE tissue samples were sectioned and placed on positively charged slides. Sections were deparaffinized, hydrated and pretreated in DAKO target retrieval solution (cat. no. S1699; Agilent Technologies, Inc.) for 20 min in a steamer. The VECTASTAIN ABC-HRP Kit, Peroxidase (Rabbit IgG) (cat. no. PK-4001; Vector Laboratories, Inc.) was used and includes the blocking serum and the 2nd HP antibody. The procedure was carried out according to the manufacturer's instructions. The slides were blocked in goat blocking serum for 30 min at room temperature, washed in phosphate buffered saline (PBS) and then incubated with rabbit anti-PBRM1 monoclonal antibody (1:50; cat. no. 38439; Cell Signaling Technology, Inc.) overnight at 4˚C. The slides were then washed in PBS and incubated with the supplied biotinylated anti-rabbit secondary antibody for 30 min at room temperature. The slides were washed in PBS and incubated with the supplied VECTASTAIN Elite ABC Reagent for 30 min at room temperature. The slides were

Table I. Patients and sample information.

							Tissue availability		
							Gene expression		
Patient ID	Age, years	Sex	AFP, ng/ml	PIVKA-II, mAU/ml	Hepatitis status	Tumor size, mm	Carcinomatoid component	Sarcomatoid component	Protein expression
P1	63	Female	4.3	30.0	-	55x45x50	Yes	Yes	Yes
P2	70	Male	3959.0	61.0	-	105[c]	Yes	Yes	Yes
P3	69	Male	2.7	22.0	C[a]	80x100x60	Yes	No	Yes
P4	72	Male	2.0	1425.0	-	65x60x60	Yes	Yes	Yes
P5	69	Female	7.8	61.0	C[a]	50[c]	Yes	No	Yes
P6	67	Male	3.0	64107.0	-	109x118x113	Yes	Yes	Yes
P7	65	Male	11.0	3.0	B[b]	32x29x38	Yes	Yes	Yes

Sarcomatoid and carcinomatoid components were obtained from formalin-fixed, paraffin-embedded samples from patients (P1-P7). Total transcriptome analysis of the P1, P2, P4, P6 and P7 matched samples was successful. However, analysis of the P3 and P5 sarcomatoid components failed due to low RNA quality. [a]Chronic hepatitis C. [b]Chronic hepatitis B, [c]Incomplete data. AFP, α-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonists II.

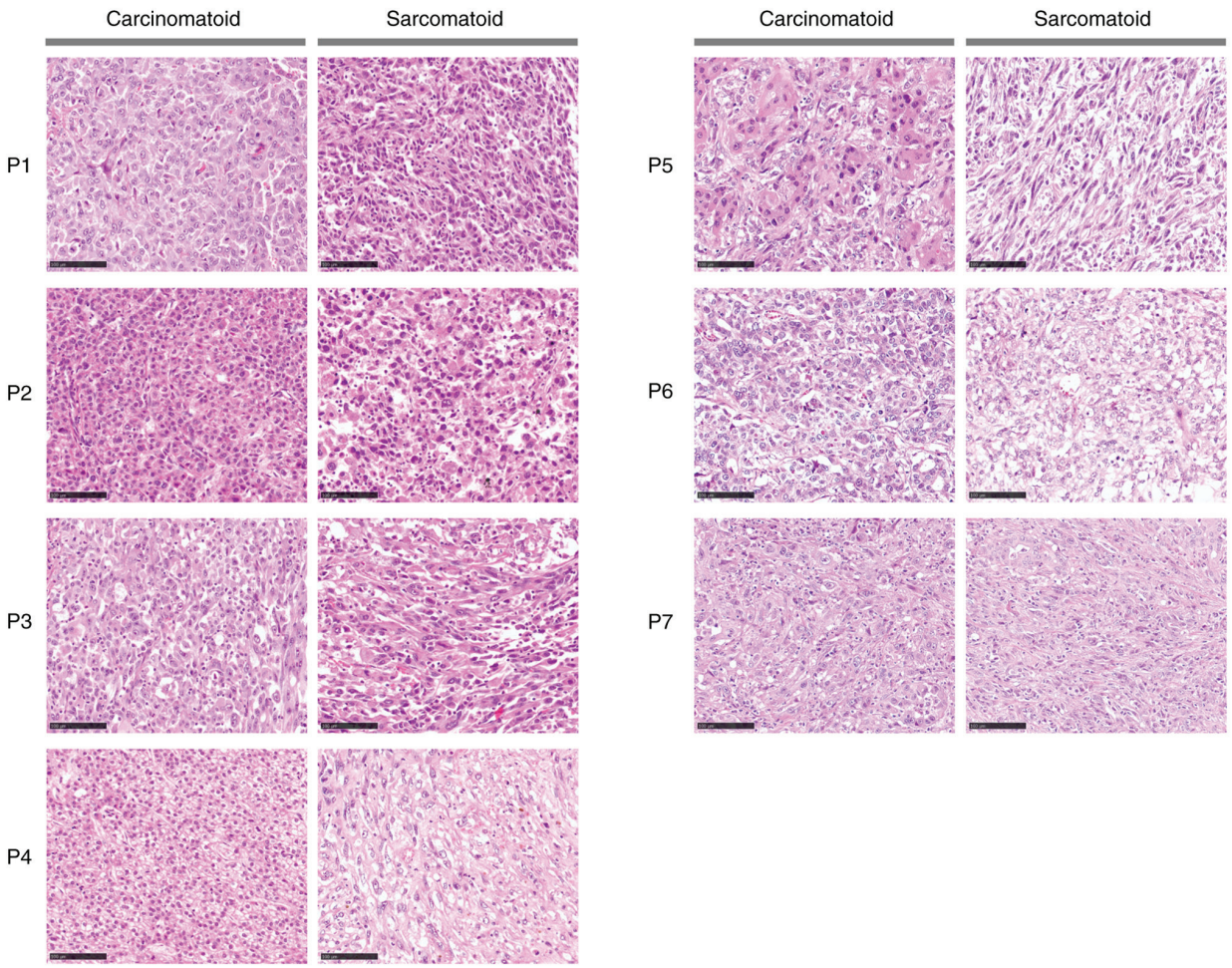

Figure 1. Hematoxylin and eosin staining of SHCC samples. Representative sarcomatoid and carcinomatoid components in the SHCC samples are shown at magnification, x20 (scale bar, 100 μm). P, patient; SHCC, sarcomatoid hepatocellular carcinoma.

washed in PBS and developed in DAB (cat. no. 8801-4965-72, Invitrogen; Thermo Fisher Scientific, Inc.) and counterstained with hematoxylin for 2 min at room temperature. Assessment of immunohistochemical staining was performed on scanned

sections captured at magnification, x10 using the Keyence BZ-X810 All-in-One light microscope. H scores were calculated for PBRM1 positivity in the sarcomatoid and carcinomatoid regions using QuPath v0.2.0-m4 image analysis software (https://qupath.github.io/) (18,19).

Statistical analysis. PBRM1 gene expression was compared by unpaired t test. Protein expression was compared using the Wilcoxon signed-rank test. All statistical analyses were performed using GraphPad Prism software (version 8.4; Dotmatics). P<0.05 was considered to indicate a statistically significant difference.

Results

Identification of differentially expressed genes in the sarcoma tissue components. A total of 7 tumor specimens, of which 5 contained sarcomatoid components and 7 contained carcinoma components, were obtained from 7 patients (Table I). Representative images of hematoxylin and eosin-stained sections showing histological differences between the sarcomatoid and carcinomatoid components in SHCC are presented in Fig. 1. Following whole transcriptome analysis of the samples, 336 genes were found to be differentially expressed between the sarcomatoid and carcinomatoid components. The top 10 downregulated and top 10 upregulated differentially expressed genes are shown in Table II. The sarcomatoid components had increased *ZRANB1* (zinc finger RANBP2-type containing 1), *IGF2BP1* (Insulin-like growth factor 2 mRNA binding protein 1), *DHRSX* (dehydrogenase/reductase X-linked), *TMEM19* (Transmembrane protein 19), *DNAJC9* (DnaJ homolog subfamily C member 9), *UBA7* (Ubiquitin-activating enzyme 7), *OR10K2* (Olfactory receptor family 10 subfamily K member 2), *DEPDC1B* (DEP domain containing 1B), *ATP10D* (ATPase phospholipid transporting 10D (putative)) and *HSPA7* (Heat shock protein family A (Hsp70) member 7 (pseudogene)) expression levels, and decreased *ADRA2B* (Adrenoceptor alpha 2B), *NHLRC1* (NHL repeat containing E3 ubiquitin protein ligase 1), *PBRM1*, *DHCR7* (7-dehydrocholesterol reductase), *SPATA7* (Spermatogenesis associated 7), *WRB* (Tryptophan-rich basic protein), *PAFAH1B1* (Platelet activating factor acetylhydrolase 1B regulatory subunit 1), *PET112*, (Glutamyl-tRNA amidotransferase subunit B), *GDA* (Guanine deaminase) and *MRPS5* (Mitochondrial ribosomal protein S5) expression levels. Moreover, visualization of the differentially expressed genes using a volcano plot indicated that decreased expression of *PBRM1* in the sarcoma component of SHCC tissues may serve as a biologically relevant marker of the sarcoma subtype (Fig. 2).

Table II. Top 10 differentially expressed genes between sarcoma and carcinoma components, listed based on the fold change.

A, Downregulated in the sarcoma component

Gene	Fold change	P-value
ADRA2B	-34.80	0.0229
NHLRC1	-30.70	0.0072
PBRM1	-29.09	0.0038
DHCR7	-25.82	0.0156
SPATA7	-21.67	0.0440
WRB	-19.56	0.0058
PAFAH1B1	-19.35	0.0130
PET112	-18.19	0.0030
GDA	-18.14	0.0337
MRPS5	-14.04	0.0349

B, Upregulated in the sarcoma component

Gene	Fold change	P-value
ZRANB1	62.08	0.0189
IGF2BP1	46.88	0.0171
DHRSX	39.15	0.0342
TMEM19	34.95	0.0178
DNAJC9	30.09	0.0258
UBA7	27.91	0.0313
OR10K2	24.94	0.0060
DEPDC1B	23.72	0.0360
ATP10D	22.88	0.0348
HSPA7	22.71	0.0263

ADRA2B, Adrenoceptor α2B; *NHLRC1*, NHL repeat containing E3 ubiquitin protein ligase 1; *PBRM1*, Polybromo 1; *DHCR7*, 7-dehydrocholesterol reductase; *SPATA7*, Spermatogenesis associated 7; *WRB*, Tryptophan-rich basic protein; *PAFAH1B1*, Platelet activating factor acetylhydrolase 1B regulatory subunit 1; *PET112*, Glutamyl-tRNA amidotransferase subunit B; *GDA*, Guanine deaminase; *MRPS5*, Mitochondrial ribosomal protein S5; *ZRANB1*, zinc finger RANBP2-type containing 1; *IGF2BP1*, Insulin-like growth factor 2 mRNA binding protein 1; *DHRSX*, dehydrogenase/reductase X-linked; *TMEM19*, Transmembrane protein 19; *DNAJC9*, DnaJ homolog subfamily C member 9; *UBA7*, Ubiquitin-activating enzyme 7; *OR10K2*, Olfactory receptor family 10 subfamily K member 2; *DEPDC1B*, DEP domain containing 1B; *ATP10D*, ATPase phospholipid transporting 10D (putative); *HSPA7*, Heat shock protein family A (Hsp70) member 7 (pseudogene).

Gene enrichment analysis of the differentially expressed genes. Next, the biological relevance of genes differentially expressed between the sarcomatoid and carcinomatoid components of SHCC was investigated. Subsequently, 336 genes were identified as differentially expressed genes between the sarcomatoid and carcinomatoid components of the tumors. Unsupervised hierarchical clustering analysis of the 336 differentially expressed genes revealed two distinct clusters, clusters I and II, which showed a strong association to the sarcomatoid and carcinomatoid components, respectively (Fig. 3A). To determine the biological relevance of the genes downregulated in the sarcomatoid components, a gene ontology analysis was performed using Metascape, and biological pathways enriched in genes from cluster II were identified. The pathways enriched in this gene cluster included 'histone methylation' and 'mitotic DNA replication checkpoint signaling', which indicated that these processes were disrupted in the sarcomatoid components (Fig. 3B).

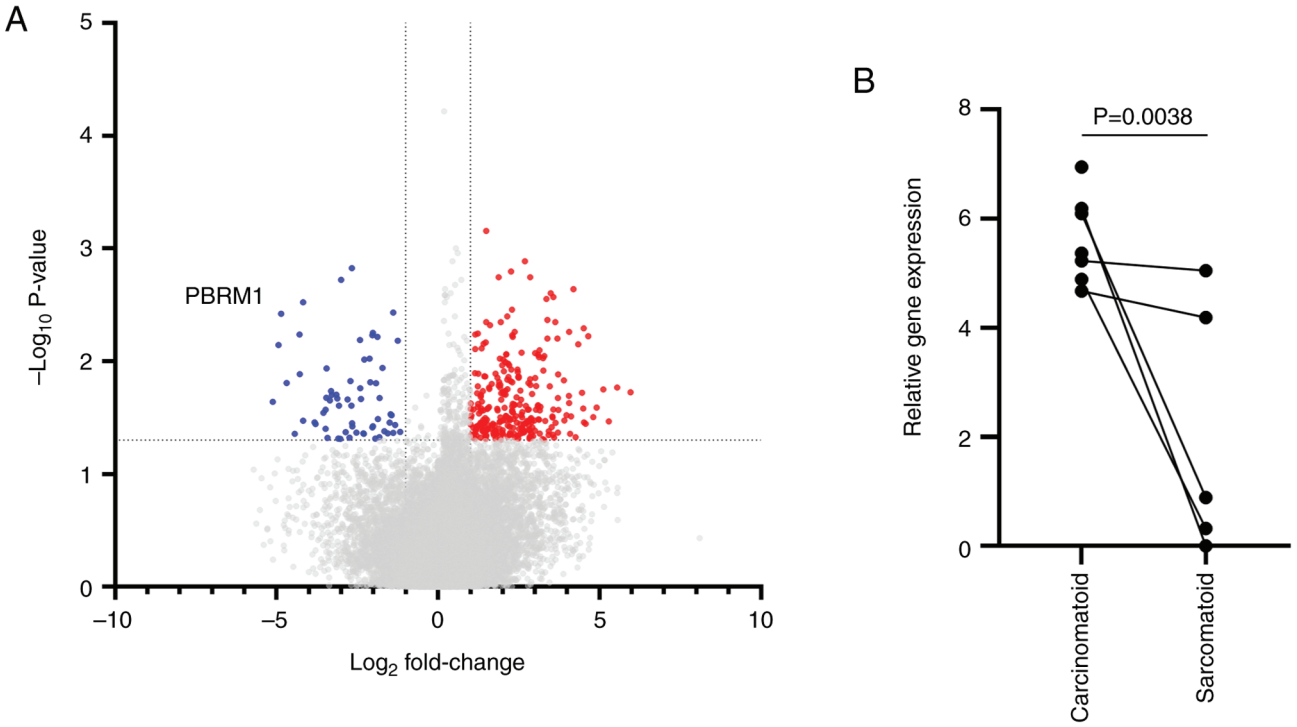

Figure 2. Differentially expressed genes between the carcinomatoid and sarcomatoid components of sarcomatoid hepatocellular carcinoma samples. (A) Volcano plot showing the differential gene expression profiles in the sarcomatoid and carcinomatoid components. Fold differences are plotted as log2 (log2 fold change) for each gene relative to its P-value ($-\log_{10}$ P-value). Genes with |fold change| >2 and P<0.05 are highlighted in red (increased expression) or blue (decreased expression). (B) Differences in the expression of *PBRM1* between the sarcomatoid and carcinomatoid components. The P-values are based on the unpaired t-test. *PBRM1*, polybromo 1.

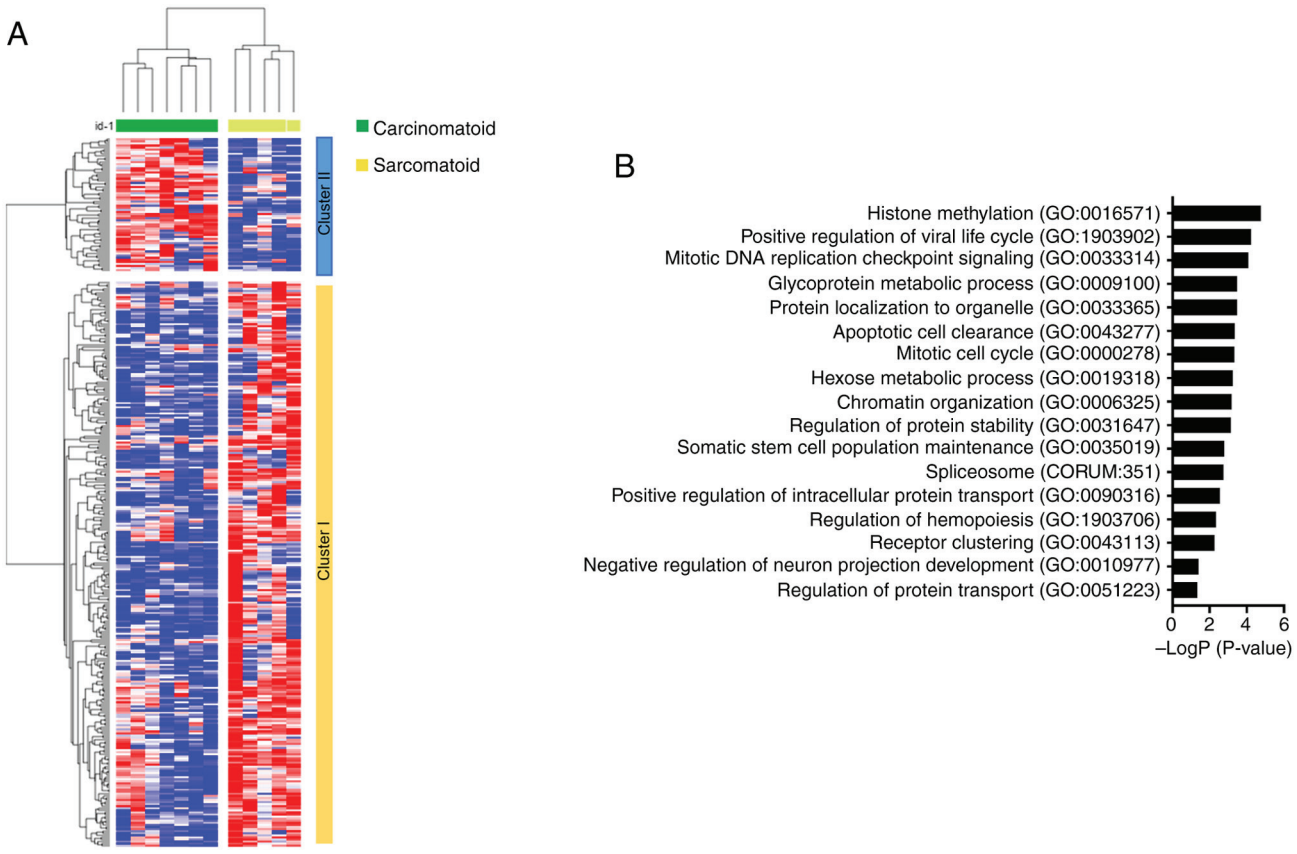

Figure 3. Gene enrichment analysis of the differentially expressed genes between the carcinomatoid and sarcomatoid components of sarcomatoid hepatocellular carcinoma samples. (A) Hierarchical clustering analysis of the 336 differentially expressed genes between the carcinomatoid and sarcomatoid components. Clustering was based on the average linkage and 1-Pearson correlation distance. (B) Plot shows the enriched biological pathways of gene cluster II from the Gene Ontology Biological Processes category analyzed using Metascape.

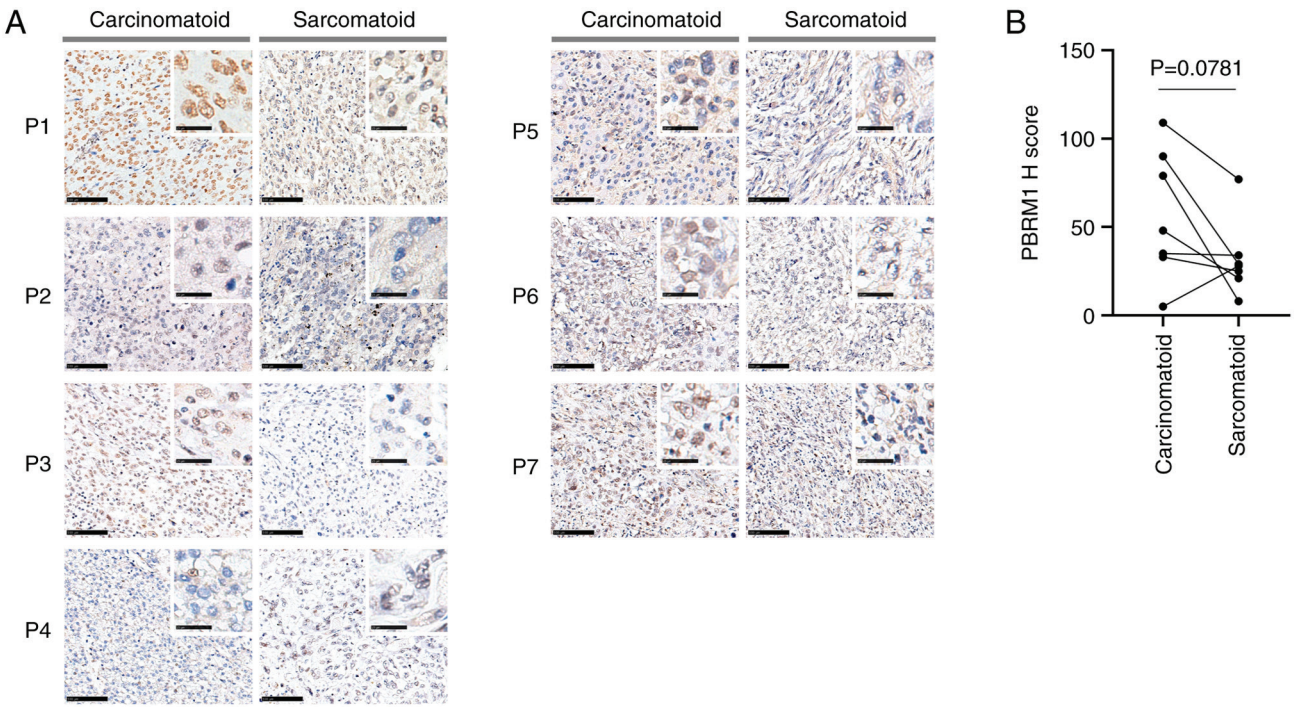

Figure 4. Immunohistochemical staining of PBRM1 in the sarcomatoid hepatocellular carcinoma samples. (A) Representative images of PBRM1 immunohistochemical staining of the sarcomatoid and carcinomatoid components; scale bar, 100 μm. Inserts are high magnification fields from each image; scale bar, 25 μm. (B) H score plot for PBRM1 expression in the sarcomatoid and carcinomatoid components. The P-value was calculated using Wilcoxon signed-rank test. P, patient; PBRM1, polybromo 1.

These results supported our hypothesis that impaired function of *PBRM1* may be associated with changes resulting in sarcomatoid differentiation.

Quantitative analysis of PBRM1 protein expression in SHCC. The aforementioned findings of the present study indicated the presence of altered *PBRM1* expression in SHCC, which indicated that *PBRM1* may serve as a marker of sarcomatoid differentiation. To evaluate the potential of *PBRM1* as a marker, PBRM1 protein expression levels in sarcomatoid and carcinomatoid components of SHCC tissues were examined by quantitative IHC. The tumors were categorized as $PBRM1^+$ when the cells had strong diffuse nuclear staining, and as $PBRM1^-$ when the cells had an absence of diffuse nuclear staining (Fig. 4A) (19). Quantitative analysis demonstrated that the PBRM1 protein expression level was markedly lower was lower in the sarcomatoid than the carcinomatoid component, although the difference was not statistically significant (P=0.0781; Fig. 4B). Quantitative analysis revealed a noticeable reduction in PBRM1 protein expression levels within the SHCC, although statistical significance was not achieved (P=0.0781; Fig. 4B). This discovery implies a potential link between diminished PBRM1 protein expression and sarcomatoid tumors, offering preliminary insights into the biological role of PBRM1 in these tumors. Nonetheless, further investigations employing larger datasets are imperative to ascertain the utility of PBRM1 in IHC as a biomarker for distinguishing sarcomatoid components.

Discussion

The present study revealed that expression of the *PBRM1* gene was reduced in the sarcomatoid differentiated tumor components of SHCC tissues, compared with the matched carcinomatoid components. *PBRM1* is the second most highly expressed gene in clear cell renal cell carcinoma (ccRCC) after the *von Hippel-Lindau tumor suppressor* gene, and it is located on the short arm of chromosome 3 (3p). Truncating mutations in *PBRM1* are found in 41% of ccRCC cases, and genetic alterations in *PBRM1* have been detected in 4% of HCC cases included in The Cancer Genome Atlas database (20). Several previous studies have suggested that a loss of PBRM1 protein may be a potential biomarker of RCC and associated with adverse pathological factors and poor patient prognosis in this disease (19,21,22). However, to the best of our knowledge, the expression status of *PBRM1* in SHCC has not been previously reported. In the present study, decreased *PBRM1* expression in the sarcomatoid components of SHCC was demonstrated, although DNA sequencing of the *PBRM1* gene in the sample cohort was unsuccessful (data not shown). Gene expression analysis using FFPE specimens failed in two components of the sarcoma-like component. of samples, suggesting the importance of high-quality specimens, such as frozen specimens. Frozen specimens are superior to FFPE samples for sequencing due to the absence of artificial alterations caused by formalin fixation. Gene mutation analysis using DNA is greatly affected by chemical modifications of nucleobases (especially C>T changes) because it analyzes base changes in sequences. On the other hand, the gene expression analysis used in this study is based on a method that amplifies a set region for each gene and estimates changes in gene expression between samples based on the number of reads obtained, and thus is considered to be less susceptible to the effects of aging degradation and artificial changes in bases due to formalin fixation than DNA analysis.

The biological significance of downregulated PBRM1 expression is largely unknown, but the gene ontology analyses of the present study suggested that chromatin-related pathways are altered in SHCC, suggesting that reduced *PBRM1* expression may affect chromatin function, given that *PBRM1* is an essential gene for chromatin remodeling (23). Other identified differentially expressed genes may also serve a role in the alteration of chromatin-related pathways in SHCC. While reduced PBRM1 expression is suggested to affect chromatin function due to its role in chromatin remodeling, it is common for multiple genes to collaborate or interact in complex biological processes. Other differentially expressed genes identified in the study could potentially contribute to the alteration of chromatin-related pathways through various mechanisms, such as transcriptional regulation, protein-protein interactions, or downstream signaling pathways. Moreover, this finding was consistent with the molecular functions of *PBRM1*. PBRM1 is part of a protein associated with cell proliferation, and its role is of particular interest in cancer; PBRM1 is a component of the BAF complex (Brahma associated factor complex), which is involved in chromatin remodeling and regulation of gene expression (24). Mutations or deletions in *PBRM1* are associated with some types of cancer, particularly renal cell carcinoma, and may affect the abnormal cell growth of cancer cells (25). Therefore, *PBRM1* is considered one of the genes that play an important role in cancer research. It has also been reported to be involved in cell proliferation, such as being a regulator of *TP53*, which plays a central role in the cell cycle check mechanism (26), or *PBRM1* being regulated by *TP53* to become a critical regulator of p21 (27,28). Further research and functional studies would be needed to determine the specific roles of these genes and how they collectively impact chromatin function in SHCC. Functional deletion of *PBRM1* has been previously reported to be associated with an upregulation of IL-6 and its downstream molecules, such as TNFα, which act on surrounding T cells to stimulate cancer immunity (29). In particular, JAK-STAT3 is also known to be involved in interferon signaling (30,31), which appears to be relevant to kidney cancer treatments because interferon therapy, which exhibits immunomodulatory, anti-angiogenic, and direct antitumor activity, has been used as a treatment for HCC (32). Loss of PBRM1 function suppresses the expression of β2-microglobulin (33), which is required for cancer antigen presentation, thereby reducing the efficacy of immune checkpoint inhibitor therapy. However, additional studies involving more samples are needed to validate these findings.

The present study has limitations, including its retrospective design, the absence of blood sample analysis, and a small sample size. Blood samples play a crucial role in diagnosis, and the absence of their analysis in this study hinders clinical application. To confirm the present findings and advance the development of a simplified diagnostic method for SHCC, future studies should involve larger sample sizes and incorporate blood sample analysis. Another limitation of the present study is that the RNA-seq results could not be confirmed by reverse transcription-quantitative PCR. This is, due to the retrospective nature of the present study, because frozen or freshly isolated tissues were not available. A further limitation of the present study is an absence of normal tissue samples, again due to its retrospective nature. In 4 selected cases, whole transcriptome analysis was performed using RNA extracted from a small region of normal cells in the same tissue section that the pathologist determined to be normal hepatocytes. However, evaluable data was obtained from only 1 case, and therefore, the results of the normal tissue analysis were not included in the present study.

The present study demonstrated that *PBRM1* expression status may serve as a diagnostic marker for SHCC when assessed through gene expression analyses or IHC. However, it's important to note that these findings should be interpreted with caution, as the study did not compare the results with normal tissues, and the IHC results comparing the sarcomatoid and carcinomatoid components were not statistically significant. Further validation and comparison with normal tissues would be necessary to confirm the utility of PBRM1 as a diagnostic marker for SHCC. The expression of *PBRM1* in SHCC was associated with the protein expression level determined by IHC (34), and this could be used to develop tools for routine diagnosis and potential treatment of SHCC. A histological diagnosis of HCC is not mandatory when the imaging diagnosis of HCC is clear. However, the presence of a sarcomatoid component in the tumor tissue is unclear in most patients with HCC. PBRM1 may aid in the diagnosis of SHCC and enable a uniform diagnosis. SHCC has a poorer prognosis than typical hepatocellular carcinoma and may require different therapeutic approaches. Uniform diagnosis of the presence of sarcomatoid features by PBRM1 (e.g., immunostaining) will lead to analysis of the mechanisms underlying SHCC, help provide a more accurate prognosis for all patients with sarcomatoid HCC, and allow better-informed decisions regarding patient treatment. Future clinical performance studies using receiver operating characteristic curve analyses are necessary to further establish the predictive value of PBRM1 expression for the diagnosis of sarcomatoid components.

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Availability of data and materials

The data generated in the present study are not publicly available due to privacy considerations. Genomic data consisting of DNA or RNA sequences obtained from tumor cells from individual patients that contain >40 loci are considered an 'individual identification code' under the Guideline for Personal Information Protection Act in Japan (14). The RNA-seq data in the present study are considered as personal information as they contain >40 loci. Consent for public release of sequence data as an individual identification code has not been obtained from the 7 patients of the present study. Therefore, the public release of these data were not allowed. However, the datasets used and/or

analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TY, MK and KN designed and supervised the research. MI, ST, SK, TN, HM, KT and MS collected the samples and clinical information. KS and KN analyzed the data. KN and MADV performed the statistical analysis. TY, KS and KN prepared the figures and tables. KS and KN wrote the manuscript. TY and KS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The Institutional Ethics Review Boards of Kansai Medical University Hospital (Hirakata, Japan; approval no. 2019074), Osaka Metropolitan University Hospital (Osaka, Japan; approval no. 2020-185) and Kindai University Faculty of Medicine (Osaka-Sayama, Japan; approval no. 31-201) approved the present study. All patients provided written informed consent for participating in the study and allowed the collection of tumor tissue specimens for analysis.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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