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The immunohistochemical combination of low SGLT2 expression and high PRDX4 expression independently predicts shortened survival in patients undergoing surgical resection for hepatoblastoma

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Abstract

Background Hepatoblastoma (HB) is the most common malignant solid tumor of the liver in children and is a fatal disease with a poor prognosis. Therefore, indicators that can be used for the early prediction of the HB prognosis are necessary. Sodium glucose cotransporter 2 (SGLT2) is a glucose transporter protein present in the proximal renal tubules. Studies have shown that SGLT2 is associated with the occurrence of tumors and is upregulated in various tumors. Peroxiredoxin 4 (PRDX4) is an antioxidant enzyme with a secretory function and is located in the cytoplasmic endoplasmic reticulum. Recent reports have suggested that it is closely related to the development and prognosis of various cancers. To some degree, this is highly suggestive of the interplay between SGLT2 and PRDX4.

Methods In the present study, clinical data and post-surgical paraffin-embedded specimens from 75 HB patients were collected, and hematoxylin and eosin and immunohistochemical staining of SGLT2 and PRDX4 were used to analyze their expression and correlation with the clinicopathological features and prognosis.

Results We found that low SGLT2 and high PRDX4 expression predicted a significantly shorter survival and worse clinical condition in HB patients. Furthermore, when low SGLT2 expression was combined with high PRDX4 expression, the event-free survival and overall survival were significantly reduced. Univariate and multivariate Cox proportional hazards analyses showed that low SGLT2 and high PRDX4 expression in HB were independent prognostic factors for the survival after surgical resection.

Conclusion The immunohistochemical combination of low SGLT2 and high PRDX4 expression can independently predict a poor prognosis in HB patients.

Keywords Hepatoblastoma, Sodium glucose cotransporter 2 (SGLT2), Peroxiredoxin 4 (PRDX4), Clinicopathology, Prognosis

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Background

Hepatoblastoma (HB) is a fatal disease that usually occurs in children and is characterized by rapid growth, strong invasiveness, and distant metastasis [1]. After receiving standardized treatment, the 5-year overall survival (OS) rate is as high as 80%, but the prognosis of some children in the advanced stage is poor [2]. The use of molecular markers to predict the occurrence and development of malignant tumors has become a hot topic in clinical research. Therefore, there is an urgent need to find a reliable molecular marker to predict the prognosis of children with HB, which can facilitate determining the clinical diagnosis and treatment.

Sodium glucose cotransporter 2 (SGLT2) is a member of the sodium-glucose cotransporter family. It reabsorbs glucose from the urine into renal tubular epithelial cells by active transport to maintain a stable level of glucose in the blood [3]. SGLT2 is normally expressed only in the proximal tubules of the kidney. However, SGLT2 expression has been demonstrated in various types of cancer cells, such as hepatocellular carcinoma, pancreatic cancer, prostate cancer, intestinal tumor, lung cancer, breast cancer, and brain tumors [4–10]. SGLT2 inhibitors block the ability of cancer cells to take up glucose, and glycolysis plays a central role in tumor metabolism and growth, as reflected by the high glucose uptake rates [11]. This activity aids the tumor cell survival and proliferation under adverse conditions. SGLT2 expression in different tumor cells confers sensitivity to therapy-induced cell death. Therefore, SGLT2 overexpression often indicates tumor cell proliferation and a poor efficacy of antitumor drugs. However, its role in HB has not been clearly demonstrated. We believe that SGLT2 has the potential to be a useful indicator of the prognosis and survival in patients with HB.

The peroxiredoxin (PRDX) family, which includes six members, is a ubiquitous antioxidant protein with catalase activity [12]. These molecules are widely present in organisms and mainly affect the biological behavior of tumor cells by participating in multiple signaling pathways related to reactive oxygen species (ROS) [13]. Domestic and international studies have found that PRDXs are upregulated in a variety of tumors, and their expression is closely related to tumor proliferation, invasion, and metastasis [14–18]. In the PRDX family, PRDX4 has an extracellular secretion function and may serve as a biomarker for many diseases (such as type 2 diabetes and atherosclerosis) [19, 20]. However, more interesting to our group is the role of PRDX4 in malignant tumors, especially HB, which presently remains unclear. Our previous studies confirmed that PRDX4 is involved in the occurrence and development of hepatoblastoma. Metastasis and differentiation were observed in the group with a high expression of PRDX4 [21]. Other reports have found that PRDX4 overexpression is associated with a poor prognosis in a variety of cancers [22–24]. Although the molecular mechanisms vary among different types of cancer, overexpression of PRDX4 may indicate proliferation, migration, and invasiveness of cancer cells. However, its mechanism of action in HB is not yet fully understood. In addition, since PRDX4 has an extracellular secretory function, we believe that PRDX4 can serve as an important indicator concerning the prognosis of HB and may be a potential therapeutic target.

Although the direct connection between SGLT2 and PRDX4 in HB has not been clearly confirmed, it can be speculated that they may interact through mechanisms such as mutual regulation of metabolic-redox state, joint regulation of glucose metabolism and antioxidant response, and cross-talk of related signaling pathways. SGLT2 may enhance the glycolysis process of tumor cells by promoting glucose uptake, thereby increasing the generation of intracellular ROS [25]. At this time, PRDX4 can protect tumor cells from oxidative damage by clearing excessive ROS. SGLT2 may also activate certain downstream signaling pathways by affecting glucose metabolism [26], which may also regulate the expression or activity of PRDX4. Therefore, SGLT2 and PRDX4 may be used as indicators for judging the prognosis of HB. This study mainly explored the expression of SGLT2 and PRDX4 proteins in HB tissues and their relationship with pathological characteristics and prognosis, aiming to provide new ideas for clinical diagnosis and treatment.

Materials and methods

Tissue samples

The present study used surgical specimens from HB patients ($n=75$) collected from Kanazawa Medical University Hospital. The specimens were obtained from HB patients who underwent surgery at the above medical institution from 2000 to 2018 and had pathological reports and follow-up data available.

The exclusion criteria were as follows: (1) perioperative death (defined as death during the initial hospitalization or within 30 days after surgery), (2) other concurrent malignancies, and (3) current medical problems severe enough to shorten life expectancy. All resected specimens were formalin-fixed and paraffin-embedded, and histopathological features were examined by four pathologists (Y.L., A.S., T.O., and S.Y.). We did not find any discrepancies in the evaluations among the four pathologists. We adopted the International Pediatric Liver Tumor Consensus Classification as a histological classification guideline [27]. The event-free survival (EFS) referred to the time from randomization to the first occurrence of any of the following events: disease progression not amenable to surgical treatment, local or distant recurrence, or death from any cause. The OS was defined as the time from

randomization to death from any cause. Patients were followed up and prospectively evaluated every month within the first postoperative year and then at approximately two-to three-month intervals using abdominal computed tomography (CT), magnetic resonance imaging (MRI), and serum alpha-fetoprotein (AFP). CT and MRI were performed every six months for three years after surgery. All metastatic disease completely removed by surgery and/or chemotherapy. Additional examinations were performed if symptoms or signs of recurrence were observed. This study was approved by the Ethics Committee of Kanazawa Medical University (No. I367). Among the 75 HB patients, most of them (71/75, 94.67%) received the cisplatin-based chemotherapy and the specimens were collected after the initial chemotherapy; only 4 samples were collected without initial chemotherapy.

Reagents and instruments

SGLT2 staining was performed using a Leica Bond-Max automatic dyeing machine (Leica, Buffalo Grove, IL, USA) and Bond Polymer Refine Detection kit. SGLT2 immunohistochemistry (IHC) staining was performed using an SGLT2 rabbit polyclonal antibody (ab85626, diluted 1:100; Abcam Inc., Cambridge, MA, USA). The IHC procedure for PRDX4 (PA5-85252, dilution 1:1000; Thermo Fisher Scientific Inc.) was performed manually. The secondary antibody was obtained from Nichirei Biosciences Inc. (Histofine Simple Stain MAX-PO424152).

Histopathological and IHC staining and result interpretation

Hematoxylin and eosin (H&E) staining was performed to confirm the HB diagnosis. The IHC procedure involved the following: 1) deparaffinization and rehydration; 2) 0.5% hydrogen peroxide blocking for 15 min at room temperature; 3) antigen retrieval in trypsin solution; 4) primary antibody staining overnight at 4°C; 5) secondary antibody staining for 30 min at room temperature; and 6) 3, 3' diaminobenzidine (DAB) imaging and hematoxylin counter staining. H&E and IHC staining images were captured and analyzed quantitatively using the NanoZoomer Digital Pathology Virtual Slide Viewer software program (Hamamatsu Photonics Corp., Hamamatsu, Japan). The positive controls were human kidney specimens for SGLT2 and human PRDX4 transgenic mouse pancreas for PRDX4. The negative controls involved omitting the primary antibodies for SGLT2 and PRDX4. IHC expression was evaluated by Histological score (H-score) and receiver operating characteristic curve (ROC) cruve (Supplementary Material 1). H-score was scored by combining staining intensity and the percentage of positive tumor cells. Staining intensity was usually scored on a scale of 0–3, where 0 was no staining, 1 was weak staining, 2 was moderate staining, and 3 was strong staining; The percentage of positively stained tumor cells were classified by intervals ranging from 0 to 100%. The calculation formula for H-score is ∑(Intensity score×Percentage of positive tumor cells). The H-score ranges from 0 to 300. We define low expression as less than 100 and high expression as greater than 100 based on the expression of SGLT2 and PRDX4.

Statistical analysis

A statistical software program (Statistical Package for the Social Sciences 27.0) was used for data processing and analyses. The chi-square test was used to analyze the correlation between the expression of SGLT2 and PRDX4 and clinicopathological characteristics. The Kaplan-Meier method and a multivariate Cox regression analysis were used to evaluate prognostic factors. $P<0.05$ was considered to indicate significance.

Results

Clinical data

The clinical and pathological data of the 75 HB patients are shown in Table 1. The age of the patients ranged from 0 to 13 years old, including 65 patients 0 to 3 years old, 5 patients 3 to 7 years old, and 5 patients ≥8 years old, with an average age of 2.04 years old. There were 50 males and 25 females; 26 cases with a maximum tumor diameter of <10 cm and 49 cases with a maximum tumor diameter of ≥10 cm; 2 cases with serum AFP concentration (ng/ml)<1000, 55 cases with 1000-1000000, and 18 cases with >10,000,000; 26 cases with pretreatment extent of disease stage (PRETEXT) I/II, 30 cases with PRETEXT III and 19 cases with PRETEXT IV (not equivalent to metastasis); 19 cases with metastasis and 56 cases without metastasis; 37 cases with fetal type, 8 cases with embryonic type, 3 cases with mixed epithelial type, 25 cases with mixed epithelial and mesenchymal type, and 2 cases with other types in histological classification; 15 patients with recurrence within 5 years; and 6 patients who died within 5 years.

Expression of SGLT2 and PRDX4 in HB specimens

The HB tumor cells were obviously atypical, with eosinophilic cytoplasm, a desmoplastic reaction, and inflammatory cell infiltration by H&E staining in low power (Fig. 1A, C). High-magnification H&E staining showed that the tumor cells were highly atypical, with abundant eosinophilic cytoplasm, prominent nucleoli, and increased nuclear-cytoplasmic ratio (Fig. 1B, D). The expression of SGLT2 and PRDX4 was observed in HB specimens and benign liver tissues (very weak staining pattern in the latter, Supplementary Material 2), and both staining patterns were cytoplasmic. The tumor treatment response did not affect the expression pattern of SGLT2 and PRDX4 and there were no significant changes. We

Table 1 The clinicopathological characteristics of the HB patients

Characteristics	Patients ($n=75$)
Sex	
Female	25
Male	50
Age (years)	
0–3	65
3–7	5
≥8	5
Maximal tumor diameter (cm)	
< 10 cm	26
≥ 10 cm	49
Serum AFP (ng/ml)	
< 1000	2
1000-1000000	55
> 1,000,000	18
PRETEXT stage	
I/II	26
III	30
IV	19
Metastasis	
Absent	56
Present	19
Histological category	
Fetal	37
Embryonal	8
Mixed epithelial	3
Epithelial and mesenchymal	25
Others	2
Recurrence within 5 years	
Absent	60
Present	15
Death within 5 years	
Absent	69
Present	6

divided the patients into the low-SGLT2 group (Fig. 1E, F), high-SGLT2 group (Fig. 1G, H), high-PRDX4 group(Fig. 1I, J), and low-PRDX4 group (Fig. 1K, L).

Expression of SGLT2 and PRDX4 and the correlation with clinicopathological features

Among the 75 HB patients, 44 (58.7%) were in the low-SGLT2 group, and 31 (41.3%) were in the high-SGLT2 group. A chi-square test analysis showed that SGLT2 expression was not significantly related to clinical pathological factors (Table 2), although there was no significant difference in the EFS between the low and high-SGLT2 groups ($P=0.167$, Fig. 2A). However, the OS of the low-SGLT2 group was significantly worse than that of the high-SGLT2 group ($P=0.024$, Fig. 2B). There were 41 cases (54.7%) in the low-PRDX4 group and 34 (45.3%) in the high-PRDX4 group. A chi-square test analysis showed that the expression of PRDX4 was significantly related to death within 5 years (Table 2); In Kaplan-Meier analysis, the EFS and OS of the high-PRDX4 group were significantly worse than those of the low-PRDX4 group ($P=0.043$, 0.027, Fig. 2C, D).

Relationship between the combined expression of SGLT2 and PRDX4 and clinical pathological characteristics

Based on the expression of SGLT2 and PRDX4, the patients were divided into high-SGLT2+high-PRDX4 groups (High-High group), high-SGLT2+low-PRDX4 groups (High-Low group), low-SGLT2+high-PRDX4 groups (Low-High group) and low-SGLT2+low-PRDX4 groups (Low-Low group). From the pathological data and statistical analysis results, the High-High group was significantly related to PRETEXT stage and metastasis, the High-Low group was significantly related to recurrence within five years, the Low-High group was significantly related to death within 5 years, and no correlation with clinicopathological characteristics were found in the Low-low group (Table 3). In the Kaplan-Meier analysis, the EFS and OS of the Low-High group was significantly worse than those of the other groups (Fig. 3A, B). On comparing the four groups (Low-Low group, Low-High group, High-Low group, and High-High group), the EFS and OS of the Low-High group were significantly worse than those of the other groups (Fig. 3C, D).

Results of the survival analysis of 75 HB patients

The endpoint of the follow-up of patients with HB was death or the survival to the last deadline. The results of the Kaplan-Meier univariate analysis showed that the patient's EFS was related to age, PRETEXT stage, metastasis, histological category, SGLT2, and PRDX4, and the difference was statistically significant ($P<0.05$). A Cox multivariate analysis showed that PRETEXT stage, metastasis, and PRDX4 were the main factors affecting the patient's EFS (Table 4). It is worth mentioning that when low SGLT2 was combined with high PRDX4, a univariate analysis found that it was associated with the EFS, but a multivariate analysis did not find this result (Table 4). The patient's OS was related to the PRETEXT stage, metastasis, SGLT2, and PRDX4, and the difference was statistically significant ($P<0.05$). A Cox multivariate analysis showed that PRETEXT stage, metastasis, SGLT2, PRDX4, and SGLT2 combined with PRDX4 were the main factors affecting the patient's OS (Table 5). In addition, when low SGLT2 was combined with high PRDX4, both univariate and multivariate analyses found that it was associated with the OS (Table 5).

Discussion

Increased intracellular glucose levels have been observed to help improve the cancer cell survival in many cancers [28–31]. When the body's demand for glucose

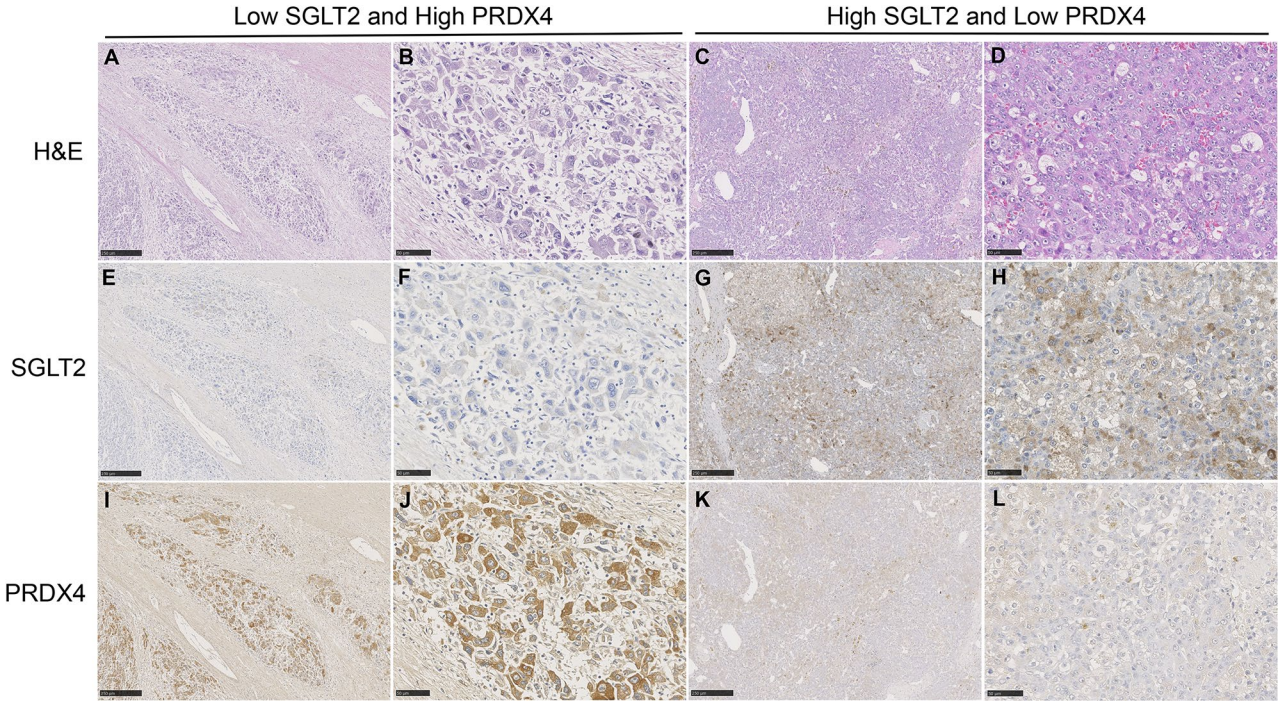

Fig. 1 The representative histological and immunostaining images of the Low-High (low SGLT2 and high PRDX4) group and High-Low (high SGLT2 and low PRDX4) group in same areas. (**A**, 100×, scale bar: 250 μm) H&E staining of the Low-High group showed obvious atypia of tumor cells in this area, eosinophilic cytoplasm, profibrotic reaction and inflammatory cell infiltration. (**B**, 400×, scale bar: 50 μm) Under high power, it can be seen that the tumor cells have obvious nuclear pleomorphism, abundant eosinophilic cytoplasm, and obvious nucleoli. (**C**, 100×, scale bar: 250 μm) H&E staining of the High-Low group showed that tumor cells were arranged in nests, with eosinophilic cytoplasm and a small amount of pigmentation. (**D**, 400×, scale bar: 50 μm) High magnification showed that the tumor cells have significant atypia, obvious nucleoli, and an increased nucleocytoplasmic ratio. (**E**, 100×, scale bar: 250 μm) Low magnification area of low SGLT2 expression, with most tumor cells showing no staining. (**F**, 400×, scale bar: 50 μm) In the area of low SGLT2 expression under high magnification, a small number of tumor cells can show weak staining in the cytoplasm. (**G**, 100×, scale bar: 250 μm) In the area of high SGLT2 expression under low magnification, strong intracytoplasmic staining can be seen in most tumor cells. (**H**, 400×, scale bar: 50 μm) In the area of high SGLT2 expression under high magnification, strong intracytoplasmic staining can be seen in most tumor cells. (**I**, 100×, scale bar: 250 μm) In the area of high PRDX4 expression under low magnification, diffuse and strong staining in the cytoplasm of tumor cells can be seen. (**J**, 400×, scale bar: 50 μm) In the area of high PRDX4 expression under high magnification, diffuse and strong staining in the cytoplasm of tumor cells can be seen. (**K**, 100×, scale bar: 250 μm) Low-power PRDX4 expression area, with a small number of tumor cells showing staining. (**L**, 400×, scale bar: 50 μm) In the area of low expression of PRDX4 under high magnification, a small number of tumor cells showed weak staining in the cytoplasm

metabolism exceeds the supply of the local vascular system, glucose deficiency will occur in the developing tumor, and glucose deficiency in the body may be a key event in the occurrence of HB [32]. In the present study, HB patients with low SGLT2 expression have a reduced ability to transport glucose, resulting in a weakened renal ability to reabsorb glucose, which leads to an increase in blood sugar levels and promotes HB growth. We confirmed that SGLT2 is an independent determinant, and patients with low SGLT2 expression have a worse EFS and OS than high SGLT2 expression, and the incidence of related adverse events (recurrence within 5 years and death within 5 years) tends to increase, which are significantly associated with a poor prognosis in HB. However, low SGLT2 expression is rare in other malignant tumors. Iwai et al. found that high SGLT2 expression was common in advanced lung adenocarcinomas with aggressive biological behavior [8]. Fujiyoshi et al. recently demonstrated that Cisplatin-resistant hepatoblastoma cells exhibited upregulated SGLT2 expression and activated glucose uptake to survive under cisplatin stress [33]. We speculate that this may be the target organ of these studies were not liver or most of the HB patients included in our study did not develop cisplatin resistance, and the glucose metabolism levels in different target organs in the body are also different. However, it is certain that SGLT2 can be used as a marker for the prognosis of malignant tumors.

Our research group recently reported that PRDX4 expression plays different roles in various cancers. Shioya et al. showed that low PRDX4 expression was predictive of the prognosis of patients with early lung adenocarcinoma [34]. Guo et al. also reached the same conclusion that hepatocellular carcinoma specimens with low PRDX4 expression had higher oxidative stress levels than high PRDX4 expression and a highly malignant phenotype, which was associated with a reduced OS [35]. However, the situation was slightly different for HB. Our

Table 2 Detailed correlations between different SGLT2 and PRDX4 expression levels and clinicopathological variables

Items	SGLT2		*p* value	PRDX4		*p* value
	Low (*n* = 44)	High (*n* = 31)		Low (*n* = 41)	High (*n* = 34)	
Sex						
Female	18	7	0.097	14	11	0.870
Male	26	24		27	23	
Age (years)						
0–3	38	27	0.996	36	29	0.155
3–7	3	2		4	1	
≥ 8	3	2		1	4	
Maximal tumor diameter (cm)						
< 10 cm	17	9	0.389	15	11	0.701
≥ 10 cm	27	22		26	23	
Serum AFP (ng/ml)						
< 1000	1	1	0.655	1	1	0.229
1000-1000000	34	21		27	28	
> 1,000,000	9	9		13	5	
PRETEXT stage						
I/II	18	8	0.074	15	11	0.438
III	19	11		18	12	
IV	7	12		8	11	
Metastasis						
Absent	36	20	0.090	33	23	0.203
Present	8	11		8	11	
Histological category						
Fetal	22	15	0.470	23	14	0.371
Embryonal	6	2		2	6	
Mixed epithelial	2	1		1	2	
Epithelial and mesenchymal	12	13		14	11	
Others	2	0		1	1	
Recurrence within 5 years						
Absent	33	27	0.197	33	27	0.908
Present	11	4		8	7	
Death within 5 years						
Absent	39	30	0.201	41	28	0.005
Present	5	1		0	6	

previous research showed that PRDX4 promotes the migration of hepatoblastoma cells and induces their differentiation [21]. Han et al. showed that patients with high PRDX4 expression in pancreatic cancer had a poor prognosis than low PRDX4 expression and suggested that PRDX4 may be a clinical prognostic indicator for pancreatic cancer [36]. Results of this study showed that PRDX4 expression was significantly associated with death within five years, and the high-PRDX4 group had a shorter EFS and OS than the low-PRDX4 group, and the incidence of related adverse events (such as PRETEXT stage, metastasis, recurrence within 5 years, death within 5 years, etc.) were also higher than the low-PRDX4 group. These results indicate that patients with high PRDX4 expression are at a higher risk of clinical adverse events, such as metastasis, than low PRDX4 expression, which may be related to undetected micro-metastasis leading to disease recurrence and a poor prognosis [37]. We hypothesized that high expression of PRDX4 can upregulate the level of extracellularly secreted PRDX4 and reduce oxidative stress in the microenvironment around cancer cells, keeping it within a range suitable for proliferation. Simultaneously, intracellular PRDX4 creates an antioxidant cellular environment to ensure tumor progression [13]. In this study, HB patients with high PRDX4 expression had a worse prognosis, and we have reason to believe that PRDX4 can serve as an important target for predicting the prognosis of HB.

In this study, the High-High group was significantly associated with PRETEXT stage and metastasis, the High-Low group was significantly associated with recurrence within 5 years, and the Low-High group was significantly associated with death within 5 years. These results indicate that SGLT2 and PRDX4 play an important role

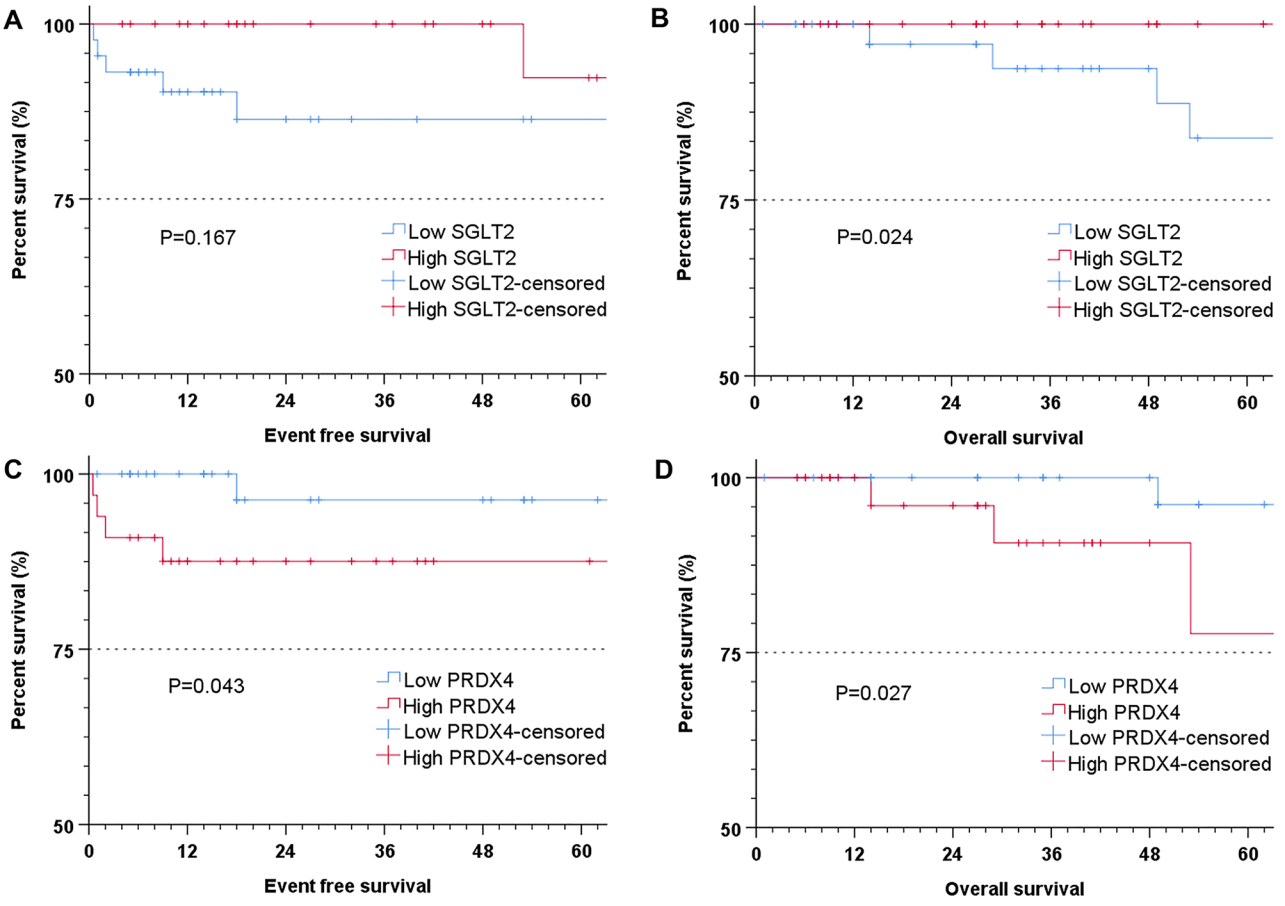

Fig. 2 A Kaplan-Meier analysis of low-expression and high-expression groups of SGLT2 and PRDX4 in HB. (**A**) There was no significant difference in the EFS between the low- and high-SGLT2 groups ($P=0.167$). (**B**) The OS of the low-SGLT2 group was significantly worse than that of the high-SGLT2 group ($P=0.024$). **C**, **D**. The EFS and OS of the high-PRDX4 group were significantly worse than those of the low-PRDX4 group ($P=0.043$, 0.027)

in the different clinical outcomes of HB. High expression of SGLT2 and PRDX4 may promote tumor metastasis and late progression, while the combination of High-Low and Low-High may affect the recurrence or survival mode of tumor cells. In addition, it should be emphasized that when low expression of SGLT2 and high expression of PRDX4 were combined, the patients' EFS and OS were significantly shortened. The results of the survival curve of the Low-High group further confirmed the previous speculation. The results of univariate analysis showed that the patients' EFS and OS were related to PRETEXT stage, metastasis, SGLT2, PRDX4, and Low-High group; Cox multivariate analysis showed that PRETEXT stage, metastasis, and PRDX4 were the main factors affecting the patients' EFS and OS. It can be seen that the prognostic factors of PRETEXT stage, metastasis in HB, and SGLT2, PRDX4, can be used as key prognostic factors in the prognosis of HB. Overall, SGLT2 and PRDX4 may affect the clinical behavior of tumors by regulating the metabolism, antioxidant capacity and proliferation potential of tumor cells. This demonstrates that the combined effect and clinical benefit of SGLT2 and PRDX4 can help stratify patients with HB and predict the prognosis. In addition, a multivariate analysis showed that PRDX4 was the independent main factor affecting patients' EFS, while SGLT2 and PRDX4 were the independent main factors affecting patients' OS.

Due to the potential intrinsic connection between SGLT2 and PRDX4, we suggest comprehensive evaluation of these two factors in HB to more accurately assess the prognosis. Most patients in this study received cisplatin-based chemotherapy, however, drug resistance remains an inevitable problem. Using SGLT2 and PRDX4 as combined prognostic markers to evaluate the prognosis of HB is of great significance for precision treatment. Through the combined detection of these markers, the biological characteristics of HB can be better evaluated, the response to chemotherapy can be predicted, and individualized treatment plans can be formulated. This stratified approach can not only optimize treatment decisions, but also improve patients' survival and quality of life.

However, several limitations associated with the present study warrant mention. This study was a retrospective and relatively small sample study and could not

Table 3 Detailed correlations between different SGLT2 and PRDX4 expression levels and others of the clinicopathological variables

Items	Groups		*p* value	Groups		*p* value	Groups		*p* value	Groups		*p* value
	High-high (*n* = 15)	Others (*n* = 60)		High-low (*n* = 15)	Others (*n* = 60)		Low-high (*n* = 18)	Others (*n* = 57)		Low-low (*n*-25)	Others (*n* = 50)	
Sex												
Female	3	22	0.221	4	21	0.797	8	17	0.251	10	15	0.386
Male	12	38		11	49		10	40		15	35	
Age (years)												
0–3	14	51	0.510	12	53	0.510	15	50	0.076	23	42	0.256
3–7	0	5		2	3		0	5		2	3	
≥ 8	1	4		1	4		3	2		0	5	
Maximal tumor diameter (cm)												
< 10 cm	3	23	0.182	5	21	0.903	8	18	0.317	9	17	0.864
≥ 10 cm	12	37		10	39		10	39		16	33	
Serum AFP (ng/ml)												
< 1000	1	1	0.524	0	2	0.524	0	2	0.066	1	1	0.428
1000-1000000	10	45		10	45		17	38		16	39	
> 1,000,000	4	14		5	13		1	17		8	10	
PRETEXT stage												
I/II	4	22	0.033	4	22	0.753	7	19	0.625	10	16	0.417
III	3	27		7	23		8	22		11	19	
IV	8	11		4	15		3	16		4	15	
Metastasis												
Absent	8	48	0.034	11	45	0.894	15	41	0.332	20	36	0.453
Present	7	12		4	15		3	16		5	14	
Histological category												
Fetal	6	31	0.820	10	27	0.356	7	30	0.332	13	24	0.966
Embryonal	2	6		0	8		4	4		2	6	
Mixed epithelial	1	2		0	3		1	2		1	2	
Epithelial and mesenchymal	6	19		5	20		5	20		8	17	
Others	0	2		0	2		1	1		1	1	
Recurrence within 5 years												
Absent	11	49	0.470	15	45	0.030	15	45	0.685	18	42	0.221
Present	4	11		0	15		3	12		7	8	
Death within 5 years												
Absent	15	54	0.202	14	55	0.831	14	55	0.011	25	44	0.071
Present	0	6		1	5		4	2		0	6	

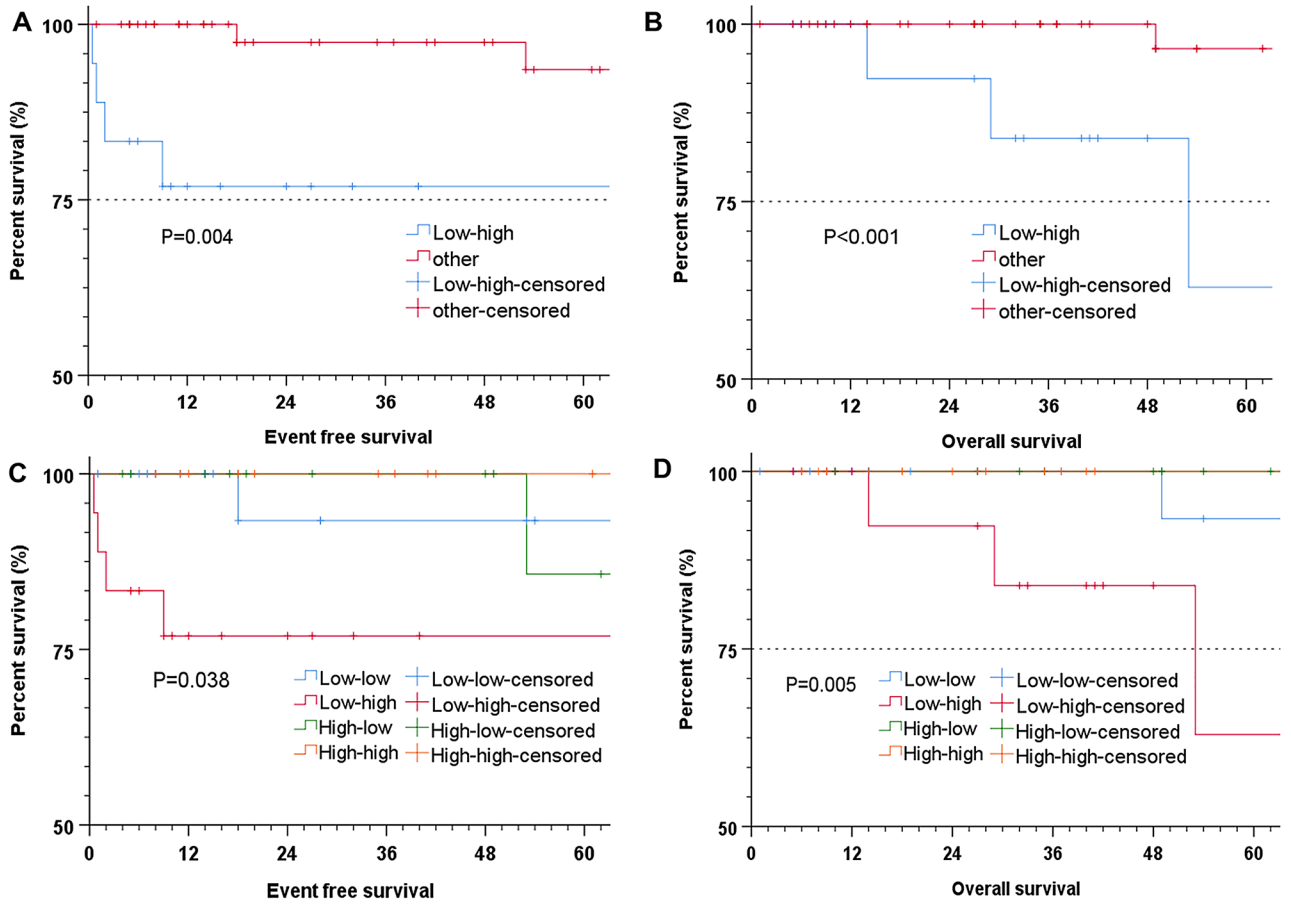

Fig. 3 A Kaplan-Meier analysis of different combinations of SGLT2 and PRDX4 expression in hepatoblastoma. (**A**, **B**). The EFS and OS of the Low-High group (low SGLT2 and high PRDX4) were significantly worse than those of the other groups ($P = 0.004$, < 0.001). (**C**, **D**). Among the four groups (Low-Low group, Low-High group, High-Low group, and High-High group), the EFS and OS of the Low-High group were significantly worse than those of the other groups ($P = 0.038$, 0.005)

comprehensively analyze the role of the two factors in the occurrence and development of HB. The underlying molecular mechanisms were not analyzed in this study and will need to be investigated in future studies. Further in-depth follow-up in much larger cohorts of HB patients, along with detailed molecular investigations using HB cell culture lines, will be required to confirm the intriguing correlation of low SGLT2 and high PRDX4 expression with recurrence and subsequent poor survival in HB patients. We suggest SGLT2 and PRDX4 be used as therapeutic targets in the study of HB, providing new therapeutic direction for HB. Although the relevant research is still in early stages, with the deepening of basic research and preclinical research, targeted treatment strategies for SGLT2 and PRDX4 may be developed in the future, which can be used alone or in combination with traditional treatment methods in the clinical treatment of HB, especially for patients with high-risk, recurrent or resistant HB.

Table 4 The univariate and multivariate analyses of EFS according to the clinicopathological variables and the expression of SGLT2 and PRDX4

	Univariate		
	Hazard ratio	**95% CI**	**p value**
Sex	4.115	0.322–52.556	0.276
Age	10.506	1.112–55.265	0.040
Maximal tumor diameter (cm)	23.210	0.679–62.270	0.081
Serum AFP (ng/ml)	3.159	1.003–7.773	0.354
PRETEXT stage	2.129	1.017–3.997	0.021
Metastasis	23.249	3.689–53.265	0.016
Histological category	3.915	1.040-14.739	0.044
SGLT2 IHC index	1.938	1.134–3.615	0.021
PRDX4 IHC index	6.828	1.513–15.157	0.016
Low-SGLT2 with high-PRDX4	7.757	1.946–18.672	0.039
	Multivariate		
	Hazard ratio	**95% CI**	**p value**
Age	9.255	0.089–43.265	0.089
PRETEXT stage	1.879	1.002–3.567	0.035
Metastasis	20.159	3.259–45.506	0.019
Histological category	2.875	0.885–13.553	0.254
SGLT2 IHC index	1.634	1.125–3.178	0.060
PRDX4 IHC index	6.828	1.218–13.219	0.033
Low-SGLT2 with high-PRDX4	8.252	1.832–16.739	0.057

Conclusions

In this study, we found that low expression of SGLT2 and high expression of PRDX4 can independently predict the poor prognosis of HB patients, and the combination of the two can more accurately assess the prognostic stratification of HB.

Abbreviations

HB	Hepatoblastoma
SGLT2	Sodium glucose cotransporter 2
PRDX4	Peroxiredoxin 4
OS	Overall survival
ROS	Reactive Oxygen Species
EFS	Event-free survival
CT	Computed tomography
MRI	Magnetic resonance imaging
AFP	Alpha-fetoprotein
IHC	Immunohistochemistry
H&E	Hematoxylin and eosin
DAB	Diaminobenzidine
ROC	Receiver operating characteristic
PRETEXT	Pretreatment extent of disease stage

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13000-025-01596-4.

Supplementary Material 1

Supplementary Material 2

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Table 5 The univariate and multivariate analyses of OS according to the clinicopathological variables and the expression of SGLT2 and PRDX4

	Univariate		
	Hazard ratio	**95% CI**	**p value**
Sex	12.313	1.233–65.357	0.966
Age	11.304	2.350-59.275	0.164
Maximal tumor diameter (cm)	16.731	1.769–55.326	0.103
Serum AFP (ng/ml)	5.342	1.529–12.370	0.338
PRETEXT stage	2.453	0.882–4.324	0.047
Metastasis	21.320	3.527–59.306	< 0.001
Histological category	6.654	2.527–16.832	0.112
SGLT2 IHC index	2.211	1.267–4.393	0.039
PRDX4 IHC index	7.447	1.745–17.432	0.006
Low-SGLT2 with high-PRDX4	8.739	3.561–23.356	< 0.001
	Multivariate		
	Hazard ratio	**95% CI**	**p value**
PRETEXT stage	2.378	0.772–4.225	< 0.001
Metastasis	22.436	3.121–54.381	0.029
SGLT2 IHC index	1.993	1.102–4.117	0.041
PRDX4 IHC index	7.779	1.643–18.305	< 0.001
Low-SGLT2 with high-PRDX4	9.675	2.343–22.765	< 0.001

Author contributions

Yao Liu, Jia Han, Xin Guo, Qian Yang, Tohru Ito, and Sohsuke Yamada conceptualized and designed the experiments; Akihiro Shioya, Yang-xian Zhang, Vu Anh Dung, and Takeru Oyama conducted the experiments; Yao Liu and Jia Han analyzed the data; Yao Liu wrote the manuscript; and Jia Han and Sohsuke Yamada edited the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

A statement on ethics approval and consent were written in the Methods section.

Consent for publication

All authors have approved the fnal version of the manuscript and have given their consent for publication.

Competing interests

The authors declare no competing interests.

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