



# SECISBP2 is a novel prognostic predictor that regulates selenoproteins in diffuse large B-cell lymphoma

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Received: 4 May 2020 / Revised: 18 September 2020 / Accepted: 18 September 2020 / Published online: 19 October 2020  
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## Abstract

The overexpression of glutathione peroxidase 4 (GPX4; an enzyme that suppresses peroxidation of membrane phospholipids) is considered a poor prognostic predictor of diffuse large B-cell lymphoma (DLBCL). However, the mechanisms employed in GPX4 overexpression remain unknown. GPX4 is translated as a complete protein upon the binding of SECISBP2 to the selenocysteine insertion sequence (SECIS) on the 3'UTR of GPX4 mRNA. In this study, we investigated the expression of SECISBP2 and its subsequent regulation of GPX4 and TXNRD1 in DLBCL patients. Moreover, we determined the significance of the expression of these selenoproteins in vitro using MD901 and Raji cells. SECISBP2 was positive in 45.5% (75/165 cases) of DLBCL samples. The SECISBP2-positive group was associated with low overall survival (OS) as compared to the SECISBP2-negative group ( $P = 0.006$ ). Similarly, the SECISBP2 and GPX4 or TXNRD1 double-positive groups ( $P < 0.001$ ), as well as the SECISBP2, GPX4, and TXNRD1 triple-positive group correlated with poor OS ( $P = 0.001$ ), suggesting that SECISBP2 may serve as an independent prognostic predictor for DLBCL (hazard ratio (HR): 2.693,  $P = 0.008$ ). In addition, western blotting showed a decrease in GPX4 and TXNRD1 levels in *SECISBP2*-knockout (KO) MD901 and Raji cells. Oxidative stress increased the accumulation of reactive oxygen species in *SECISBP2*-KO cells (MD901;  $P < 0.001$ , Raji;  $P = 0.020$ ), and reduced cell proliferation (MD901;  $P = 0.001$ , Raji;  $P = 0.030$ ), suggesting that *SECISBP2*-KO suppressed resistance to oxidative stress. Doxorubicin treatment increased the rate of cell death in *SECISBP2*-KO cells (MD901;  $P < 0.001$ , Raji;  $P = 0.048$ ). Removal of oxidative stress inhibited the altered cell death rate. Taken together, our results suggest that SECISBP2 may be a novel therapeutic target in DLBCL.

**Supplementary information** The online version of this article (<https://doi.org/10.1038/s41374-020-00495-0>) contains supplementary material, which is available to authorized users.

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## Introduction

Malignant lymphoma is the most common neoplasm, accounting for more than 50% of all hematological malignancies [1], with more than 34,000 cases diagnosed in Japan [2]. Meanwhile, diffuse large B-cell lymphoma (DLBCL) is the most common (25–35%) and aggressive forms of malignant non-Hodgkin lymphoma in adults [3]. DLBCL comprises a large number of disparate subtypes distinguished by morphology, phenotype, gene expression profiles, and clinical outcomes [3]. Specifically, DLBCL patients overexpressing glutathione peroxidase 4 (GPX4), an intracellular antioxidant enzyme, have poor prognosis and overall survival (OS). These GPX4-overexpressing cells are reportedly resistant to reactive oxygen species (ROS)-induced cell death, and thus, accounts for a more aggressive form of DLBCL. However, the regulation of GPX4 expression remains to be characterized [4].

*GPX4*, one of 25 selenoproteins found in humans, contains selenocysteine (Sec; the 21st amino acid), and functions in

Sec-dependent redox reactions [5, 6]. Thioredoxin reductase 1 (*TXNRD1*) is another selenoprotein that is a flavin-containing NADPH-dependent oxidoreductase with an active site containing a redox-active disulfide bond [7]. Selenoproteins employ a unique mechanism for incorporating Sec in the place of UGA termination codon during translation [6, 8–16]. Sec is transported to the ribosome by selenocysteine-specific tRNA (Sec-tRNA) containing the anticodon complementary to the UGA codon. The 3' untranslated region (UTR) of selenoprotein mRNAs, called Sec Insertion Sequence (SECIS) element, is where the SECIS-binding protein 2 (*SECISBP2*) inserts Sec upon recognizing the UGA codon. However, the expression level of *SECISBP2* in DLBCL and the regulation of *GPX4* remain to be fully elucidated. Here, we hypothesized that *SECISBP2* regulates downstream selenoprotein levels and serves as a promising novel therapeutic target. This study, therefore, aimed at investigating the biological role of *SECISBP2* in DLBCL. B-cell lymphoma cell lines were employed to understand the correlation between *SECISBP2* and *GPX4* and *TXNRD1*.

## Materials and methods

### Patients and pathological specimens

We examined pathological specimens obtained from 165 patients with DLBCL at the Tokyo Medical and Dental University Hospital, Tokyo, between 2004 and 2015 in addition to the patient cohort from previous study [4]. All patients were subjected to R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) based therapy as induction therapy. Recurrence of DLBCL after initial R-CHOP induction was observed in 42 cases (25.4%), of which 26 cases (61.9%) were treated with salvage chemotherapy, and 13 cases (31.0%) were treated with salvage chemotherapy and peripheral blood stem cell transplantation. Pathologic diagnosis was confirmed by two pathologists (MK and KY) based on the criteria set by the World Health Organization. Specimens were obtained by biopsy or surgical resection, fixed in 10% neutralized formalin, and embedded in paraffin according to routine protocols used in conventional histopathological examination. Informed consent was obtained from all the patients and the study was approved by the ethics committees of Tokyo Medical and Dental University. All procedures were performed in accordance with the ethical standards established by these committees (M2000-1818).

### Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues were sectioned at a thickness of 4  $\mu$ m, placed on silane-coated slides, and deparaffinized. Heat-based antigen retrieval, endogenous

peroxidase blockade using 3% hydrogen peroxide, and blocking were performed with normal sera. The sections were incubated overnight with primary antibodies against SECISBP2 and TXNRD1 (Table S1) at 4 °C. Primary antibodies were detected using the ABC kit (Vector Laboratories, Burlingame, CA, USA). Color development was performed using diaminobenzidine (Nichirei Bioscience, Japan) or the HISTOFINE simple stain AP series (Nichirei Bioscience). We performed new cases of GPX4, CD20, CD10, bcl-6, and MUM-1 as the same condition of the previous study [4]. CD20 expression was used for diagnosis of DLBCL. CD10, bcl-6, and MUM-1 expression were used to categorize DLBCL into germinal center B (GCB) and non-GCB groups based on the Hans algorithm [17].

### Clinicopathologic analysis

The following nine parameters were evaluated as clinicopathologic factors: age ( $\geq 65$  vs.  $< 65$  years), gender (male vs. female), Ann Arbor status (stages I and II vs. III and IV), lactate dehydrogenase levels (normal;  $< 210$  IU/l vs. elevated;  $\geq 210$  IU/l), performance status (0–1 vs. 2–4), B symptoms (yes vs. no), number of extranodal sites (0 vs.  $\geq 1$ ), bone marrow involvement (yes vs. no), and Hans algorithm (GCB vs. non-GCB type) [17].

### Cell lines and culturing conditions

MD901 cells (DLBCL cell line) were obtained from the Department of Hematology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan. Raji cells (Burkitt lymphoma cell line) were purchased from the American Type Culture Collection (Manassas, VA). These cells were cultured in RPMI-1640 medium supplemented with L-glutamine and phenol red (Wako Pure Chemical Industries, Ltd, Osaka, Japan) containing 10% fetal bovine serum and 1% penicillin–streptomycin. Cells were passaged at a ratio of 1:5–1:8 every 2–3 days.

### Establishment of *SECISBP2*-knockout MD901 and Raji cells

Two monoclonal antibodies were obtained from each of the *SECISBP2*-KO cell lines (*SECISBP2*-KO1-1, KO1-2, KO2-1, and KO2-2) and control CRISPR vector-transfected MD901 and Raji cells (Ctrl 1, Ctrl 2) using limiting dilutions. The detailed methods are described in Supplementary information.

### Analysis of ROS by flow cytometry in *SECISBP2*-KO cells

CellROX® Deep Red Reagent (Thermo Fisher Scientific) detected intracellular ROS in MD901 and Raji cells. This

reagent freely enters cells and fluoresces after being oxidized by ROS [18, 19]. The cells were analyzed by flow cytometry using the BD FACSCanto™ II analyzer (Becton Dickinson and Company, Franklin Lakes, NJ, USA). The experimental details are provided in Supplemental methods.

### Assessment of cell viability after doxorubicin treatment

Viability of the *SECISBP2*-KO and control MD901 and Raji cells after doxorubicin treatment (LKT Labs, Inc) was analyzed by flow cytometry. Moreover, to investigate the cell condition without oxidative stress, the cells were cultured with doxorubicin and ferrostatin-1 (Fer-1; inhibitor of oxidative stress-induced cell death; SML0583, Sigma-Aldrich). The experimental details are provided in Supplemental methods.

### Statistical analysis

Correlations between two groups were assessed using the Fisher's exact test. OS was determined from the date of diagnosis and that of last follow-up, or death. Kaplan–Meier survival curves were used to estimate rates of OS. The Log–rank test was used to analyze differences in survival between groups. Univariate and multivariate analyses were performed using the Cox proportional hazard regression model.  $P \leq 0.05$  was considered statistically significant. Results between all *SECISBP2*-KO cells (KO1-1, KO1-2,

KO2-1, KO2-2) and all Ctrl cells (Ctrl 1, Ctrl 2) were compared since, although the sequence of gRNA and the mutation caused differed, all *SECISBP2*-KO cells lacked *SECISBP2*, and were thus considered as one group. Meanwhile, results were obtained independently in triplicate for each experiment. All statistical analyses were performed using the free statistical software EZR (Version 1.41, Bone Marrow Transplantation 2013:48,452–458).

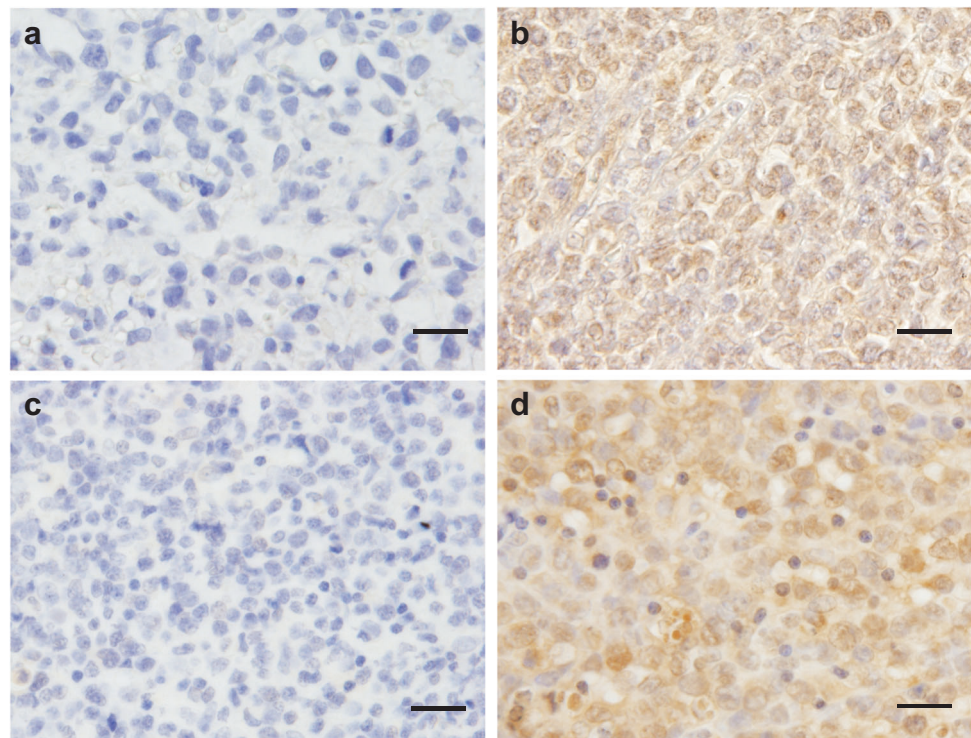
## Results

### Expression of *SECISBP2* and selenoproteins in patients with DLBCL

First, we performed immunostaining to analyze the expression of *SECISBP2* in DLBCL based, on which samples were classified as *SECISBP2*-positive or negative. Among the 165 patients, 75 cases (45.5%) were *SECISBP2*-positive (Fig. 1a, b). *SECISBP2* was detected in DLBCL cell cytoplasm. Similarly, we performed immunostaining of *TXNRD1* in the same cases and identified 88 cases (53.3%) to be *TXNRD1*-positive; *TXNRD1* was also detected in the DLBCL cell cytoplasm (Fig. 1c, d). *GPX4* was detected in 87 cases (52.7%) in this study. Control tissue staining was presented as Fig. S1.

Second, to investigate the correlation between *SECISBP2* and selenoprotein expression, we analyzed the staining pattern using the Fisher's exact test. The immunostaining of

**Fig. 1 Immunohistochemical analysis of *SECISBP2* and selenoprotein.** Representative images of sections immunostained for *SECISBP2* in patients with diffuse large B-cell lymphoma (DLBCL) (a, b) and for *TXNRD1* (c, d). All images are shown at a magnification of  $\times 400$ . Scale bars, 20  $\mu\text{m}$ . **a** A *SECISBP2*-negative DLBCL. **b** A case of DLBCL with cytoplasmic staining for *SECISBP2*. **c** A case of *TXNRD1*-negative DLBCL. **d** A case of DLBCL with cytoplasmic staining for *TXNRD1*.



**Table 1** SECISBP2, GPX4, and TXNRD1 expression via Fisher's exact test.

	GPX4		P value
	Negative	Positive	
<b>SECISBP2</b>			
<b>Negative</b>	55	35	<b>&lt;0.001</b>
<b>Positive</b>	23	52	
	<b>TXNRD1</b>		
<b>SECISBP2</b>			
<b>Negative</b>	56	34	<b>&lt;0.001</b>
<b>Positive</b>	21	54	

SECISBP2 and GPX4 were independent and positively correlated (Table 1a,  $P < 0.001$ ). Similarly, the immunostaining of SECISBP2 and TXNRD1 were independent and positively correlated (Table 1b,  $P < 0.001$ ).

### Clinicopathologic significance of SECISBP2 and selenoprotein expression in DLBCL

The correlation between clinical outcome and SECISBP2 and selenoprotein (GPX4 and TXNRD1) expression in DLBCL was investigated based on various clinical features. SECISBP2-positive cells correlated with Ann arbor staging classification ( $P = 0.013$ ; Table S2). For selenoprotein, GPX4 expression was correlated with age ( $P = 0.044$ ), which agrees with results in previous study [4], and TXNRD1 expression correlated with age ( $P = 0.001$ ) and LDH ( $P = 0.020$ ) (Table S2).

### Prognosis of patients classified by immunohistochemistry

The correlation between SECISBP2 expression and patient prognosis was then assessed. Kaplan–Meier curves analysis revealed that the SECISBP2-positive groups were associated with poor OS as compared to the SECISBP2-negative group (Fig. 2a,  $P = 0.006$ ). We then performed univariate and multivariate analysis on nine parameters, and SECISBP2 expression using the Cox proportional hazard model (Tables 2 and 3). SECISBP2 expression was an independent predictor of shorter OS (HR: 2.223,  $P = 0.036$ ; Table 3). Furthermore, we analyzed double- or triple-positive cases to determine the correlation between the SECISBP2 expression and that of each selenoprotein. The SECISBP2 and GPX4 double-positive group (52 cases) also showed shorter OS ( $P = 0.001$ ) along with SECISBP2 and TXNRD1 double-positive group (54 cases;  $P < 0.001$ ; Fig. 2b, c). Finally, SECISBP2, GPX4, and TXNRD1 triple-positive group (39 cases) was also showed shorter OS ( $P = 0.010$ ; Fig. 2d, HR: 2.693,  $P = 0.008$ ; Table 3).

### SECISBP2 regulates the expression of selenoproteins in malignant lymphoma cells

Based on the abovementioned results, we speculated that SECISBP2 levels regulate selenoprotein expression. We generated *SECISBP2*-KO as well as control MD901 and Raji cells (Ctrl) and performed western blotting to measure the GPX4 and TXNRD1 levels. GPX4 and TXNRD1 expression was reduced in *SECISBP2*-KO MD901 and Raji cells (Fig. 3a). However, the reduction of selenoproteins was lower than that expected based on the immunostaining results, especially slight in Raji cells.

### SECISBP2-KO increases oxidative stress accumulation in malignant lymphoma cells

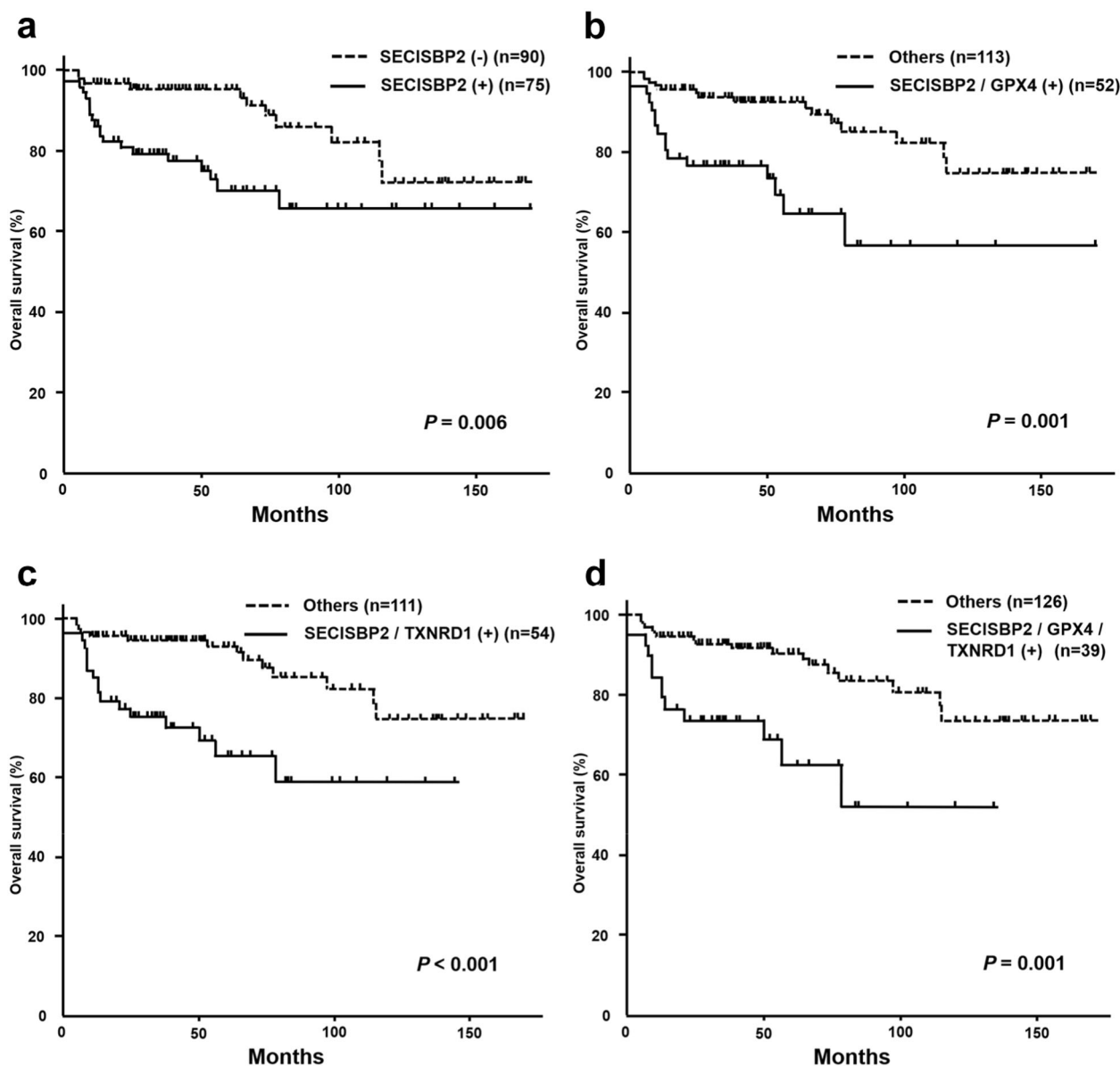
Selenoproteins help ameliorate oxidative stress [20–22]. Thus, if SECISBP2 regulates selenoproteins, we predicted that knocking out of *SECISBP2* would reduce tolerance to oxidative stress and induce ROS-induced cell death. To visualize oxidative stress accumulation, we used ROS detection system for cells treated with tert-butyl hydroperoxide (TBHP, a major inducer of oxidative stress) and Fer-1. The fluorescent signal between TBHP and both TBHP and Fer-1 was higher in *SECISBP2*-KO cells as compared to counterparts in Ctrl cells, indicating an increased accumulation of ROS in *SECISBP2*-KO cells (Fig. 3b, c, MD901;  $P < 0.001$ , Raji;  $P = 0.020$ ). Furthermore, *SECISBP2*-KO and Ctrl cells were cultured without Fer-1 and analyzed for cell proliferation under oxidative stress. *SECISBP2* knockout significantly reduced cell proliferation at 72 h (Fig. 3d, MD901;  $P = 0.001$ , Raji;  $P = 0.030$ ). Results for each cell line were presented in Fig. S2.

### SECISBP2-KO enhance the cell death effect of doxorubicin

To investigate if SECISBP2 could be a therapeutic target for malignant lymphoma, we determined the rate of cell death upon doxorubicin treatment (drug included in R-CHOP therapy). *SECISBP2*-KO cells showed a significantly higher rate of cell death compared to Ctrl cells upon doxorubicin treatment at 24 h (Fig. 3e, MD901;  $P < 0.001$ , Raji;  $P = 0.048$ ). We then combined doxorubicin and Fer-1 to determine the effect of oxidative stress. The rate of *SECISBP2*-KO cell death decreased and was comparable to that of Ctrl cells, suggesting that ROS-induced cell death was impeded (Fig. 3e). The results for each cell line were presented in Fig. S2.

## Discussion

Recognition of the UGA termination codon is required for incorporation of the trace element selenium, an essential



**Fig. 2 Overall survival of patients based on the expression of SECISBP2 and selenoproteins.** Kaplan–Meier curves for the survival of diffuse large B-cell lymphoma (DLBCL) patients based on the expression of SECISBP2 and selenoproteins. **a** SECISBP2-positive group was associated with shorter overall survival (OS) than SECISBP2-negative group ( $P=0.006$ ). **b** SECISBP2 and GPX4

double-positive group showed significantly shorter OS as compared to the other group ( $P=0.001$ ). **c** SECISBP2 and TXNRD1 double-positive group showed significantly shorter OS as compared to the other group ( $P<0.001$ ). **d** SECISBP2, GPX4, and TXNRD1 triple-positive group showed significantly poorer prognosis in OS than the other group ( $P=0.001$ ).

constituent of the 25 selenoproteins identified in humans [17]. Although the detailed mechanism involved in the uptake of selenium has not been characterized, its incorporation depends on the interacting factor of the SECIS sequence and Sec-tRNA [9–16, 21–25]. Moreover, the binding of SECISBP2 to the eukaryotic Sec-specific elongation factor (EFsec) and 60S ribosomal subunit plays an important role in the interaction between SECIS sequence and Sec-tRNA [8, 9, 21, 26]. Meanwhile, our previous study showed that GPX4 overexpression in malignant

lymphoma resulted in the poor prognosis of patients; however, GPX4 mRNA levels did not change. Thus, GPX4 expression may be regulated at the translational level or via a post-translational mechanism, e.g., using the ubiquitin-proteasome system [4].

The depletion, or deletion, of *SECISBP2* decreased the levels of selenoproteins, in mouse hepatocytes [27, 28], HEK293T cells [13] and human mesothelioma cells [10]. Thus, the expression of selenoprotein is highly dependent on *SECISBP2* [8, 29]. Furthermore, it has been reported that

**Table 2** Univariate analyses of clinicopathologic factors related to overall survival.

Variable	Category	No. of patients	<i>P</i> value by log-rank test
Age	≥60 years	113	0.390
	<60 years	52	
Gender	Male	91	0.820
	Female	74	
Ann Arbor stage	1.2	84	<b>0.001</b>
	3.4	81	
LDH	Normal	55	<b>0.044</b>
	Elevated	110	
Performance status	0–1	140	<b>0.002</b>
	2–4	25	
B symptoms	No	44	<b>0.004</b>
	Yes	121	
Extranodal site	0	53	0.370
	≥1	112	
Bone marrow involvement	No	143	0.160
	Yes	22	
Hans algorithm	GCB	70	0.270
	Non-GCB	95	
SECISBP2	Negative	90	<b>0.006</b>
	Positive	75	
SECISBP2/GPX4	Negative	113	<b>0.001</b>
	Positive	52	
SECISBP2/TXNRD1	Negative	111	<b>&lt;0.001</b>
	Positive	54	
SECISBP2/GPX4/TXNRD1	Negative	126	<b>0.001</b>
	Positive	39	

*LDH* lactate dehydrogenase, *GCB* germinal center B cell.

Bold values indicate statistical significance  $p < 0.05$ .

*SECISBP2* gene is biologically important. For example, mutations in *Secisbp2* gene results in a decrease in selenoprotein synthesis, and is involved in the development of multiple diseases and conditions, including azoospermia, axonal dystrophy, T lymphoproliferative disorders, insulin sensitivity [30], deafness, myopathy, mental and motor coordination [31], thyroid hormone resistance [32], and pontocerebellar hypoplasia 2D [7].

In this study, we used immunohistochemistry to demonstrate that SECISBP2 was positively correlated with the expression of two types of selenoproteins, GPX4 and TXNRD1. The expression of SECISBP2 may regulate the interaction between the SECIS sequence and tRNA-sec, recognition of the UGA codon, and efficiency of selenium uptake. Thus, SECISBP2 may positively regulate the synthesis of selenoproteins in DLBCL. The results from the in vitro malignant lymphoma cell study, also supported this hypothesis.

**Table 3** Cox proportional hazard analysis of clinicopathologic factors related to overall survival.

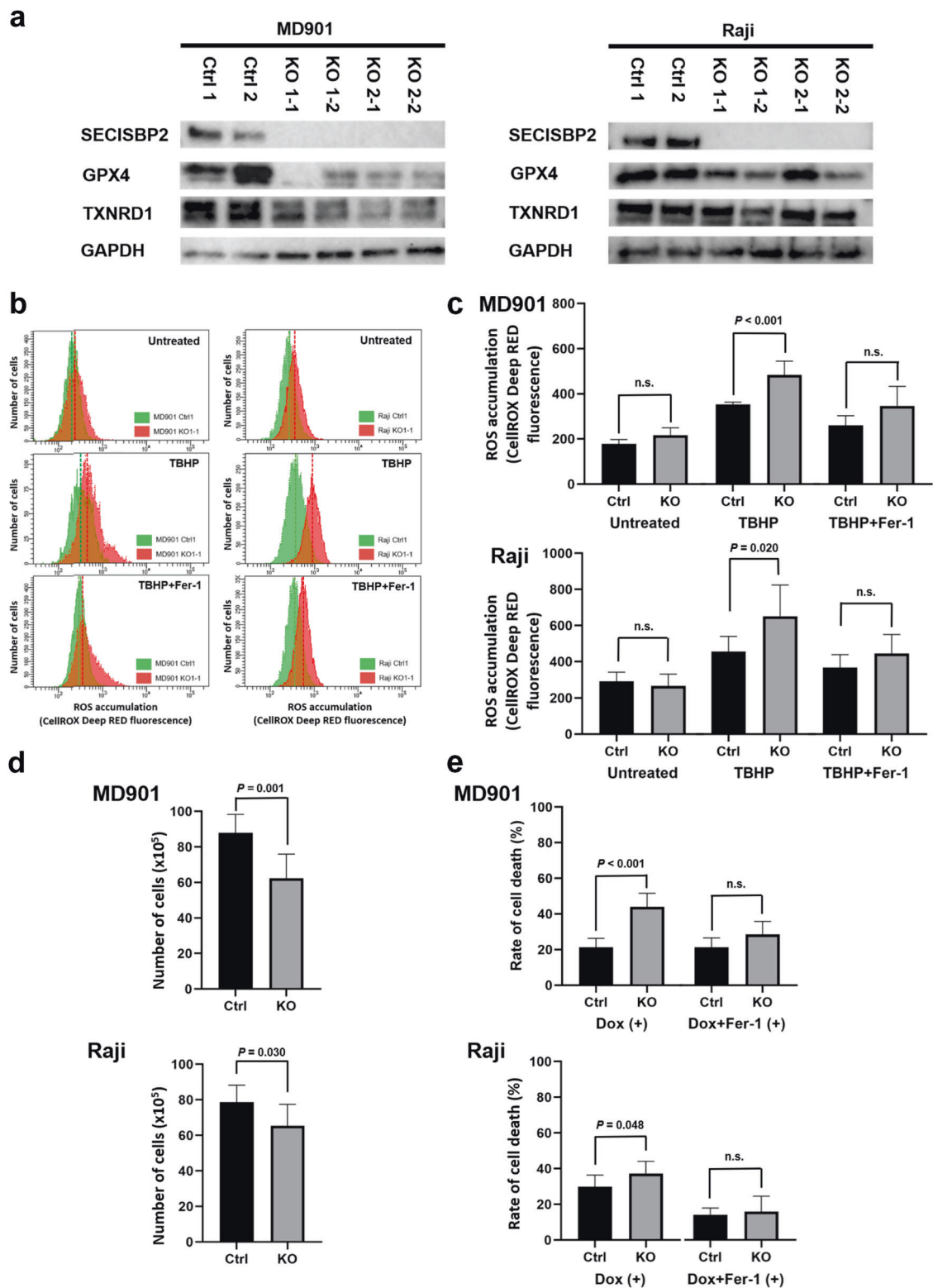
Variables	HR	95% CI	<i>P</i> value
<b>SECISBP2</b>			
Ann Arbor stage	2.102	0.852–5.188	0.107
LDH	1.379	0.554–3.434	0.489
Performance status	1.965	0.869–4.443	0.105
B symptoms	1.916	0.892–4.112	0.095
SECISBP2	2.223	1.056–4.722	<b>0.036</b>
<b>SECISBP2/GPX4/TXNRD1</b>			
Ann Arbor stage	2.022	0.820–4.987	0.126
LDH	1.348	0.536–3.434	0.526
Performance status	1.989	0.887–4.462	0.095
B symptoms	2.028	0.956–4.301	0.654
SECISBP2/GPX4/TXNRD1	2.693	1.291–5.616	<b>0.008</b>

*HR* hazard ratio, *CI* confidence interval, *LDH* lactate dehydrogenase.

Bold values indicate statistical significance  $p < 0.05$ .

We also demonstrated that the increase in ROS accumulation under oxidative stress in *SECISBP2*-KO MD901 and Raji cells resulted from decreased tolerance for oxidative stress upon reduced selenoprotein levels. Accumulation of ROS suppresses the growth of cancer cells [33] and causes tumor cell death via apoptosis or necrosis [34]. Oxidative stress exerts these different effects on tumor growth based on the cellular conditions [35]. In this study, the deletion of SECISBP2 resulted in accumulation of ROS and subsequent suppression of tumor cell growth. Furthermore, doxorubicin increased the rate of cell death in *SECISBP2*-KO cells. Hence, this may serve as a novel therapeutic strategy for controlling ROS levels in tumor cells. Moreover, SECISBP2 may serve as a promising therapeutic target for malignant lymphoma, including DLBCL resistant to standard treatment and associated with poor patient prognosis. A similar study reported that the combinatorial use of auranofin (an inhibitor of the thiorodoxin system) and buthionine sulfoximine (a rate-limiting enzyme in glutathione biosynthesis) resulted in tumor cell death in malignant B-cell lymphoma; moreover, this regimen is less toxic to normal cells [36]. Although we have not determined the effect of SECISBP2 deletion in normal cells, it can be speculated that a drug targeting SECISBP2 causes tumor cell death in DLBCL via the downregulation of selenoproteins, such as GPX4 and TXNRD1, and has higher antitumor efficacy than that of auranofin or buthionine sulfoximine.

We also observed that SECISBP2 overexpression was an independent predictor of poor prognosis. However, GPX4, TXNRD1, and each double- or triple-positive groups were also determined to be predictors of poor prognosis, which is



consistent with the regulation of selenoproteins by SECISBP2. Conversely, in renal cancer, SECISBP2 overexpression results in favorable prognosis of patients, while SECISBP2 does not effectively predict breast cancer

(analysis in The Human protein atlas [37] using The Cancer Genome Atlas data [38]). Thus, the correlation between prognosis and SECISBP2 expression differs depending on the cancer type. Nevertheless, in the current study,

◀ **Fig. 3 In vitro characteristics of SECISBP2.** The effect of SECISBP2 on cell proliferation and cell death response in malignant lymphoma (MD901 and Raji) cells. In cell line studies, the results of all *SECISBP2*-KO cells (KO1-1, KO1-2, KO2-1, KO2-2) vs. all Ctrl cells (Ctrl 1, Ctrl 2) were compared. **a** Western blotting showing the protein levels of SECISBP2 and selenoproteins in *SECISBP2*-knock-out (KO) MD901 and Raji cells. Control MD901 and Raji cells (Ctrl) were used for comparison. **b** As a typical example, we showed the levels of reactive oxygen species (ROS) in Ctrl 1 MD901 or Raji (green) and *SECISBP2*-KO1-1 MD901 or Raji (red) cells using the fluorescent dye CellROX Deep Red Reagent in flow cytometry. The score between cells treated with TBHP (inducer of oxidative stress) and treated with TBHP and Fer-1 (inhibitor of oxidative stress) showed the ROS level. The ROS level in KO1-1 cells was higher than Ctrl 1. **c** Mean fluorescence intensity of intracellular accumulation of ROS significantly increased in *SECISBP2*-KO cells treated with TBHP as compared to that in Ctrl cells (MD901;  $P < 0.001$ , Raji;  $P = 0.020$ ). **d, e** Analysis of cell proliferation without Fer-1. *SECISBP2*-KO reduced cell proliferation more than Ctrl cells at 72 h (MD901;  $P = 0.001$ , Raji;  $P = 0.030$ ) **f** The rate of cell death upon doxorubicin treatment in MD901 and Raji cells using flow cytometry. The mean rate of dead cells significantly increased upon doxorubicin treatment in *SECISBP2*-KO cells as compared to the rate of cell death in Ctrl cells (MD901;  $P < 0.001$ , Raji;  $P = 0.048$ ). Upon treatment with doxorubicin and Fer-1, the rate of dead cell in *SECISBP2*-KO cells was negated as ROS-induced cell death was reduced.; n.s. non-significant.

SECISBP2 deletion may have negatively affected tumor cell proliferation or drug resistance, thereby affecting prognosis.

Interestingly, SECISBP2 knockout abolishes the expression of selenoproteins in mouse hepatocytes [27], however *SECISBP2*-KO MD901 and Raji cells showed only partial reduction of GPX4 and TXNRD1. Previous report showed that knocking out SECISBP2 partially reduces selenoproteins translation, however some selenoproteins are translated without SECISBP2 [28]. Thus, malignant lymphoma cells may also exhibit SECISBP2-independent translation. Alternative SECIS-binding proteins such as PRL30, NCL, SECIBP2L, and EIF4A3, also affect the expression of selenoproteins [39–42]. Hence, these genes may affect the expression level of selenoproteins in conditions involving ablation of *SECISBP2* gene.

In addition, the decrease in TXNRD1 levels was less than that observed for GPX4 in MD901 and Raji cells; which may be attributed to nonsense-mediated mRNA decay (NMD) [10, 29, 43]. Stop codons are recognized as early stop codons when they are located 50 nucleotides upstream of the exon–exon junction, and then mRNA undergoes NMD [44]. In case selenium cannot be inserted in this UGA codon, mRNA with a stop codon upstream from the original position is produced. Notably, TXNRD1 has a UGA codon in the last exon and is not subjected to NMD [29]. Thus, a failed attempt at incorporating selenium may lead to a difference in mRNA stability and result in the different protein levels of TXNRD1 and GPX4. SECISBP2

has also been reported to be involved in mRNA stability [28]. However, *SECISBP2*-KO hepatocytes exhibit a reduction in the protein levels of selenoproteins while maintaining their mRNA levels [27]. Therefore, further studies are warranted to investigate the correlation between mRNA and protein levels of selenoproteins.

Although the current study does not attempt to understand the mechanisms involved in the overexpression of SECISBP2 in lymphoma cells and DLBCL, a recent report revealed that miRNAs regulate SECIBSP2 and selenoprotein expression [45]. Selenium is a component of selenoproteins and an essential element of Sec that is translated in place of the stop codon by *SECISBP2*. Hence, the amount of selenium also affects selenoprotein expression [13, 45]. However, the protein levels of SECISBP2 are unaffected by the selenium content [13]. In this study, we used a *SECISBP2*-knockout system to enable minimum effect of selenium deficiency on selenoprotein expression. However, increased uptake of selenium in lymphoma cells is essential in SECISBP2-overexpressing DLBCL and thus, better understanding this aspect of mechanism will help to develop SECISBP2 as a therapeutic target.

In conclusion, we have demonstrated that SECISBP2 positively regulates the expression of selenoproteins, including GPX4 and TXNRD1, and is associated with a poor prognosis of DLBCL patients. Furthermore, in vitro results highlighted the potential of SECISBP2 as a novel therapeutic target in malignant lymphoma. Further prospective studies on the correlation between the expression of SECISBP2 and prognosis of the subtypes of malignant lymphomas, including DLBCL, as well as further studies investigating the mechanism of selenoprotein regulation will serve to inform the development of SECISBP2 for clinical applications.

**Acknowledgements** This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 18K07063).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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