

Research Article

Nesprin1 deficiency is associated with poor prognosis of renal cell carcinoma and resistance to sunitinib treatment

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Short Title: The role of Nesprin1 deficiency in renal cell carcinoma

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Abstract

Introduction: Nuclear envelope spectrin repeat protein (Nesprin) 1 encoded by *SYNE1*, crucially regulates the morphology and functions of the cell. Mutations in the *SYNE1* gene are associated with various diseases; however, their significance in renal cell carcinoma (RCC) remains unknown. In this study, we have investigated the association of *SYNE1*/Nesprin1 with the progression and prognosis of clear cell RCC (ccRCC). **Methods:** *In silico* analyses of publicly available datasets of patients with RCC were performed. Based on the cohort data, Nesprin1 expression in nephrectomized tissue samples acquired from patients with ccRCC was analyzed using immunohistochemical staining. The invasion, migration, and proliferation of the *SYNE1*-knockdown human RCC cell lines were analyzed *in vitro*; moreover, RNA sequencing and Gene Set Enrichment Analysis were conducted to study the molecular mechanism underlying the association of *SYNE1*/Nesprin1 with prognosis of RCC. **Results:** Patients with RCC-associated *SYNE1* gene mutations exhibited significantly worse overall and progression-free survivals. Patients with Nesprin1-negative ccRCC tumors exhibit significantly poorer overall, cancer-specific, and recurrence-free survival rates than those recorded in the Nesprin1-positive group. *SYNE1* knockdown enhanced the invasion and migration of RCC cells, however, it did not influence the proliferation of cells. RNA sequencing and Gene Set Enrichment Analysis revealed that *SYNE1* knockdown significantly altered the expression of genes associated with oxidative phosphorylation. Consistently, patients with RCC exhibiting low *SYNE1* expression, who were treated with the vascular endothelial growth factor receptor inhibitor sunitinib, had worse progression-free survival. **Conclusions:** The results indicate that the expression of *SYNE1*/Nesprin1 and *SYNE1* mutations in patients with RCC are closely linked to their prognosis and responsiveness to sunitinib treatment.

Introduction

In 2020, kidney cancer, representing approximately 2% of all cancer cases, accounted for approximately 430,000 new cases and 180,000 deaths worldwide [1]. Renal cell carcinoma (RCC) comprises approximately 90% of kidney cancer cases, more than 70% of which are categorized as clear cell-type RCC (ccRCC). Recently, three strategies have been developed for treating metastatic RCC (mRCC): vascular endothelial growth factor receptor tyrosine kinase inhibitors (VEGFR-TKIs), mammalian target of rapamycin (mTOR) inhibitors, and immunotherapy with checkpoint inhibitors (ICIs) [2]. Among them, TKI monotherapy remains the first-line treatment for immunotherapy-unfit RCC patients [3]. Despite advances in chemotherapy [4], the incidence of ccRCC is rising and the associated mortality rate has remained unchanged [5]. One-third of ccRCC patients are diagnosed in the metastatic phase and their 5-year survival rate is less than 15% [6].

Nuclear envelope spectrin repeat protein 1 (nesprin1), encoded by *SYNE1*, belongs to the nesprin family and is distributed in various tissues and cells. Nesprin1 plays a crucial role in the formation of the linker of the nucleoskeleton and cytoskeleton (LINC) complex involved in the regulation of the shape and positioning of the nucleus [7]. Owing to its predominant localization in the outer nuclear membrane and other subcellular compartments [8, 9], Nesprin1 interacts with actin filaments [7] and other functional proteins, including Sad1 and UNC84 domain-containing proteins, which are involved in nuclear-cytoplasmic connections [7]. Consequently, an abnormality in the expression or functions of Nesprin1 leads to morphological and functional abnormalities of cells [7]. The *SYNE1* gene is associated with several diseases, such as autosomal recessive cerebellar ataxia [10], congenital cerebellar hypoplasia, cognitive impairment [11], arthrogryposis multiplex congenita [12], Emery Dreifuss muscular dystrophy [13], cardiomyopathy [14], and cellular senescence [15].

The potential contributions of insufficient LINC complex formation to the development and progression of cancer [16] is consistent with the association of the *SYNE1* gene mutations with various cancer types such as glioblastoma [17], oral cancer [18], lung cancer [19], gastric cancer [20], hepatocellular carcinoma [21, 22], intrahepatic mucinous cholangiocarcinoma [23], ovarian cancer [24], colorectal cancer [25], and liver cancer [21, 26]. Alterations in *SYNE1* have been identified in 36% of bladder cancer cell lines [27]. However, the correlation of Nesprin1 with the disease progression, prognosis, and drug responsiveness in patients with RCC remains partially unclear.

In this study, we aimed to study the relationship of the *SYNE1* gene and Nesprin1 with the prognosis of patients with ccRCC, and the role of Nesprin1 in the invasion and migration of RCC cells. Furthermore, the correlation of Nesprin1 with the responsiveness of RCC patients to VEGFR-TKI treatment has been explored.

Moreover, the potential significance and/or implications of this study can be included for improved impact of the section.

Methods

Human data collection

Clinicopathological datasets of 538 patients with ccRCC (Kidney Renal Clear Cell Carcinoma, The Cancer Genome Atlas [TCGA], Firehose Legacy) were obtained using cBioPortal for Cancer Genomics (www.cbioportal.org); 451 samples were used to analyze the clinical characteristics, including age, sex, laterality, clinical stage, pathological T stage, and Fuhrman grade, of patients. Cell line data were sourced from the Human Protein Atlas (<https://www.proteinatlas.org>). The *SYNE1* gene mutations were analyzed in patients with ccRCC using Gene Expression Profiling Interactive Analysis (GEPIA) software (<http://gepia.cancer-pku.cn>).

Seventy-seven patients diagnosed with ccRCC, who underwent radical or partial nephrectomy at the Hiroshima University Hospital between 2002 and 2012, were included in this study. ccRCC tissues that were pathologically diagnosed based on the 2016 WHO classification [28] were obtained, along with the corresponding clinical data. Written informed consent for basic and clinical research was obtained from each patient before their participation. This study was conducted following the Ethical Guidelines for Human Genome/Gene Research of the Japanese Government. This study was approved by the Institutional Review Board of Hiroshima University Hospital (approval no. E-1800 and E-2065). Clinical and genomic data were acquired from a phase 3 clinical trial reported by Motzer et al., demonstrating the comparative efficacy of sunitinib and avelumab plus axitinib in patients with mRCC (JAVELIN RENAL 101) [29]; sunitinib and axitinib are VEGFR-TKIs, whereas, avelumab, an antibody against programmed death receptor ligand 1, is used as an ICI.

Immunohistochemical staining (IHC) assay

To elucidate the correlation between Nesprin1 expressed in tumor tissues and the prognosis of patients with ccRCC, IHC staining for Nesprin1 was performed on 77 nephrectomized tissue samples. All tissue samples were fixed using formalin and embedded in paraffin (FFPE) for preservation. IHC using anti-human Nesprin1 antibody (rabbit monoclonal IgG, Abcam, USA, ab192234, 1:1000) was

conducted following previously reported [30]. Two uropathologists independently evaluated the staining intensity of the specimens in a blinded manner and classified them as positive or negative.

Cells

Human RCC cell lines 786-O (RRID: CVCL_1051) and ACHN (RRID: CVCL_1067) were obtained from the Japanese Collection of Research Bioresources Cell Bank (Japan) and cultured using RPMI-1640 medium (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, USA) and 1% penicillin-streptomycin solution (FUJIFILM Wako Pure Chemical Corporation, Japan). The cells were cultured at 37°C in a humidified chamber maintaining 5% CO₂.

Western Blot Analysis

Western blot analysis was performed following a previously described method [31]. Total protein was extracted from 786-O and ACHN cells with RIPA buffer (FUJIFILM Wako Pure Chemical Corporation). After equalizing the protein concentrations, the samples were electrophoretically analyzed using 5–20% precast polyacrylamide gels (SuperSep Ace; FUJIFILM Wako Pure Chemical Corporation). Subsequently, the proteins were transferred to nitrocellulose blotting membranes (GE Healthcare Life Science, USA) followed by blocking with a 5% skimmed milk solution for 30 min at room temperature. Subsequently, the samples were incubated with anti-human Nesprin1 antibody (rabbit monoclonal IgG, Abcam, ab192234, 1:1000) at 4°C overnight, washed three times with TBST buffer (10 mM Tris, pH 8.0, 150 mM NaCl, and 0.05% Tween-20), and incubated with a secondary antibody (horseradish peroxidase [HRP]-conjugated anti-rabbit-IgG antibody, MBL, Japan, 1:5000) at room temperature for 1 h. Anti-beta-actin (mouse monoclonal IgG, Proteintech, USA, 1:2000) treated with secondary anti-mouse IgG-HRP (MBL, 1:5000) was used as a loading control. After another washing step, the samples were incubated with ECL liquid (Promega Corporation, USA), and Western blotting signals were detected as luminescence using X-ray film exposure.

RNA interference

To siRNA-based RNA interference-based knocking down of *SYNE1* expression in 786-O and ACHN cell lines, cells were individually transfected with two different *SYNE1*-targeting siRNAs (si*SYNE1*-1, SYNE1HSS118624; si*SYNE1*-2, SYNE1HSS177350; Stealth RNAi, Thermo Fisher Scientific) and control

siRNA (siCtrl, Silencer®, Thermo Fisher Scientific) using Lipofectamine RNAiMAX reagent (Thermo Fisher Scientific). Cells were collected for RNA and protein extraction 48 and 72 h after transfection.

Transwell invasion assay

For the Transwell invasion assay, we used 24-well plates (Corning, USA) equipped with 8.0 µm pore ThinCert™ 24 Well Cell Culture membrane inserts (Greiner Bio-One, Germany). Cells were suspended in a serum-free medium and seeded in the top chamber of the insert; serum-containing medium (10% FBS) was added to the bottom chamber. After 48 h, invasive cells that reached the bottom surface of the membrane were carefully recovered and counted using Diff-Quick staining.

Wound-healing assay

The migratory ability of cells was assessed through a wound-healing assay conducted using a wound-healing assay kit (Abcam, ab242285) following the instructions provided by the manufacturer. For this assay, cells were seeded in a culture insert (ibidi culture-insert 2 well; ibidi GmbH, Germany). After allowing cell adhesion to proceed for 48 h, the culture insert was carefully removed and washed with PBS to eliminate nonadherent cells. Subsequently, the cells were cultured using the fresh medium for 9 (786-O cells) or 12 h (ACHN cells). Cell migration areas were comparatively analyzed using ImageJ software (<https://imagej.nih.gov/ij/>) and the images were captured before and after incubation.

Cell proliferation assay

Cells (0.5×10^5 cells/well) were incubated in a 6-well cell culture plate for 72 h. The cell proliferation rate was assessed every 24 h using a Countess II FL automated cell counter (Thermo Fisher Scientific). The number of viable cells was recorded and this process was repeated three times to obtain an average value.

RNA sequencing and Gene set enrichment analysis (GSEA)

Total RNA extracted from siSYNE1- and siCtrl-transfected cells was used to generate RNA libraries using a Sure Select Strand-Specific RNA Library Preparation Kit (Agilent Technologies, USA).

Transcriptome analysis was performed using a HiSeq 2500 next-generation sequencer (Illumina). The resulting sequence tags were aligned to human genomic sequences (hg38). GSEA was performed

using the corresponding software (GSEA version 4.1.0) obtained from the Broad Institute website (<http://software.broadinstitute.org/gsea/downloads.jsp>).

Statistical analyses

One-way analysis of variance (ANOVA) with Dunnett's multiple comparison test, log-rank test, and linear regression analyses were performed using GraphPad Prism version 9 (GraphPad Software, USA). Univariate and multivariate Cox regression analyses with the calculation of hazard ratios (HR) using a Cox proportional hazards model were conducted using JMP PRO 16 software (SAS Institute Inc., USA). The risk ratio (RR) was determined using logistic regression analysis. Statistical significance was set at p values < 0.05 . Nominal (NOM) p values, false discovery rate (FDR) q values, and normalized enrichment scores (NES) were calculated using GSEA. To calculate the probability of survival, a survival period of each patient was counted from the date of surgery except for progression-free survival. Overall, cancer-specific, and recurrence-free survivals were defined as the date until death with all cases, cancer-related death, and the occurrence of any recurrence after surgery, respectively. Progression-free survival was defined as the period from the date of drug therapy administration to the first evidence of disease progression. The number of patients at risk was detected at suitable time points using the Kaplan-Meier curves.

Results

SYNE1 mutation and Nesprin 1 expression are associated with the prognosis of patients with ccRCC

We investigated the relationship between *SYNE1* mutations and the prognosis of patients with ccRCC using TCGA datasets. Among the 451 ccRCC samples, 19 *SYNE1* mutations (4.2%), comprising 15 missense, 3 truncating, and a splice site mutation, were identified (Figure S1). Patients with *SYNE1* mutations showed significantly lower rates of progression-free and overall survival than those with no *SYNE1* mutations (Figure S2); however, no significant differences in the patient characteristics were noticed between these groups (Table S1).

Among the 77 tumor tissue samples, 26 (33.8%) and 51 (66.2%) were identified as Nesprin1-negative and -positive, respectively (Figure 1a). As Nesprin1 was detectable in both Nesprin1-positive and -negative non-neoplastic renal tissues, particularly in renal tubules (Figure 1b), the absence of a Nesprin1 signal was unlikely to be caused by the loss of antibody-binding activity due to its genetic mutation. No significant differences were detected in the patient background between the groups (Table 1). The Nesprin1-negative group exhibited significantly shorter overall ($p = 0.004$), cancer-specific ($p = 0.002$), and recurrence-free ($p = 0.041$) survival than those recorded for the Nesprin1-

positive group (Figure 1c). The overall and cancer-specific survival rates were significantly correlated with the Nesprin1 expression independently of the pathological stage or Fuhrman grade (Table 2).

SYNE1 knockdown promoted invasion and migration of RCC cells

The functional influences of Nesprin1 insufficiency were analyzed using *SYNE1* knockdown cell lines (786-O and ACHN). Western blot analysis revealed that siRNAs targeting *SYNE1* substantially downregulated the expression of Nesprin1 in these cell lines compared to that in siCtrl-transfected cells (Figure 2a). The *SYNE1* knockdown cell lines, except si*SYNE1*-2-transfected ACHN cells, exhibited enhanced invasive property compared to that in the control counterparts (Figure 2b). Enhanced wound-healing activity was observed in *SYNE1*-knockdown cells compared to that in control cells, however, the effects of si*SYNE1*-1 were not statistically significant (Figure 2c). *SYNE1* knockdown did not affect the proliferation of 786-O or ACHN cells (Figure 2d).

SYNE1 knockdown induces positive enrichment of the OXPHOS and other pathways

RNA sequencing-based comparative transcriptome analysis followed by GSEA revealed information on several differentially expressed genes and indicated positive enrichment of the OXPHOS pathway in 786-O and ACHN cell lines after the introduction of both si*SYNE1*-1 and si*SYNE1*-2 than in the siCtrl-transfected counterparts (Figure 3a, b). Other pathways associated with the cell cycle, DNA repair, protein secretion, and Myc were enriched in either of the cell lines and/or by either of the siRNAs (si*SYNE1*-1 and si*SYNE1*-2) (Figure 3a).

SYNE1 expression and drug sensitivity in RCC.

Based on clinical and genomic data from the JAVELIN-RENAL 101 phase 3 clinical trial, seven hundred twenty-six patients were divided equally into three groups (each $n = 242$) according to *SYNE1* expression levels (high, medium, or low) in RCC tissues. Among them, 372 and 354 patients were treated with sunitinib monotherapy and avelumab plus axitinib combination therapy, respectively. Among sunitinib-treated patients, the rate of progression-free survival was significantly worse in the *SYNE1*-low group than in the *SYNE1*-high group (Figure 4a, left panel). However, no significant differences in survival rates were observed between different groups of patients treated with the combination of avelumab and axitinib (Figure 4a, right panel). Similar results were obtained when only PD-L1-positive cases were analyzed (Figure 4b).

Discussion

This study revealed a close relationship of *SYNE1* gene mutations and Nesprin1 expression levels with the overall, cancer-specific, and/or recurrence-free survival in ccRCC patients, suggesting

SYNE1/Nesprin1 as a potential prognostic biomarker. *SYNE1* knockdown downregulated the invasion and migration properties of RCC cells and enhanced OXPHOS responses. A positive association between *SYNE1* expression levels and the prognosis of sunitinib-treated patients with RCC was observed, regardless of PD-L1 positivity.

We identified a significantly worse prognosis in patients with ccRCC with the *SYNE1* gene mutations compared to that in cases without similar mutations. Nickerson et al. found missense mutations in the *SYNE1* gene in 9 out of 25 bladder cancer cell lines tested, however, its functional contributions were not addressed [27]. In addition to missense mutations, we identified truncating and splice site mutations in the *SYNE1* gene of patients with ccRCC using the TCGA database. Owing to the small sample size, the differential effects of different types of mutation on patient prognosis could not be elucidated in this study; this fact can be further clarified through larger cohort and/or functional studies with individual mutation-introduced cell lines.

Although the impact of each genetic mutation of *SYNE1* on the expression and/or function of Nesprin1 is unknown, we elucidated that nephrectomized patients with ccRCC without detectable Nesprin1 expression showed a poor prognosis. Consistently, *SYNE1*-knockdown 786-O and ACHN cell lines exhibited enhanced rates of invasion and migration. The cell-type-independent function of Nesprin1 was suggested by Chu et al., who demonstrated the upregulation of their invasion and migration activities in transitional and hepatocellular carcinoma cell lines after the introduction of sh*SYNE1* [21]. However, they detected significantly promoted cell proliferation after *SYNE1* knockdown, which represents an unexplained discrepancy with our results. However, the differences between si*SYNE1* and sh*SYNE1* may influence the cell phenotypes. We observed a minor difference between the effects of si*SYNE1*-1 and si*SYNE1*-2 on the invasion and migration of RCC cells.

The inconsistency between our findings and those reported by Chu et al. [21] is possibly attributed to the variability in Nesprin1-mediated intracellular signaling cascades among cell types. Nesprin1 deficiency-induced upregulation of OXPHOS-associated genes revealed through our comparative transcriptome analyses represents a novel finding. OXPHOS is a crucial cellular metabolic process; OXPHOS pathway is correlated with the efficacy of cancer treatment using VEGFR-TKIs [31, 32], and a higher risk of recurrence and death in patients with triple-negative breast cancer [33]. Furthermore, it plays a pivotal role in regulating the resistance to anti-cancer drugs, such as VEGFR-TKIs, cisplatin, MEK inhibitors, and glucose transporter1 inhibitor BAY-876 [31, 34]. Selective elimination of cancer stem cells and the inhibition of drug resistance can be achieved by targeting OXPHOS [33]. Further examination can elucidate the relative contributions of the OXPHOS pathway to cancer progression, specifically to the proliferation of RCC and other tumor cells.

Consistent with the involvement of the OXPHOS pathway in Nesprin1-regulated signaling, this study revealed the *SYNE1* downregulation-associated worse prognosis in sunitinib-treated patients with RCC. Although the overall survival rates were not included in the clinical trial data set employed, a previously identified potentially deleterious mutation in the *SYNE1* gene in a sunitinib-resistant patient with mRCC [36] further supports the link between Nesprin1 and VEGF-related signaling.

Interestingly, this correlation between *SYNE1* expression and the responsiveness to VEGFR-TKIs was inconspicuous after ICI treatment. Although a positive correlation between *SYNE1* expression and prognosis in sunitinib-treated RCC patients was observed regardless of PD-L1 positivity, the efficacy of the combined therapy using axitinib and avelumab was not affected by *SYNE1* expression in either the overall or PD-L1 positive population. Li et al. reported that *SYNE1* mutations are associated with higher tumor mutational burden (TMB) [37], which is useful in predicting the response to ICIs [37]. Therefore, low *SYNE1* expression is considered to support the high effectiveness of immune checkpoint inhibitors. The influence of *SYNE1* expression may be offset by the combined use of avelumab and axitinib, which exhibits essentially the same mode of action with sunitinib. This hypothesis can be validated through further cohort and experimental studies.

Recently, exosomes have emerged as a novel source of non-invasive tumor biomarkers. The molecular cargo in tumor-derived exosomes can also serve as biomarkers for ccRCC in the serum and urine of patients, offering valuable targets for early detection and monitoring of the disease [38]. Nesprin1 was identified in exosomes of tumor-associated myeloid-derived suppressor cells [39], suggesting the requirement of elucidating the significance of its subcellular localization for enhancing the reliability as a biomarker protein.

In conclusion, genetic mutations and expression of *SYNE1* are closely related to the prognosis of patients with ccRCC, which is associated with the regulatory role of Nesprin1 in the metastatic and invasive capacity of RCC cells. The results suggest that Nesprin1 affects the efficacy of sunitinib treatment, at least in part, through the OXPHOS pathway. Besides the recent identification of relationship between lactotransferrin expression and mTOR inhibitor responsiveness [40, 41], this study newly proposed that the expression of *SYNE1*/Nesprin1 and associated mutations are potential biomarkers for predicting the prognosis and sunitinib responsiveness of patients with RCC.

Statements

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Statement of Ethics

Study approval statement: Approval of the research protocol by an institutional review board All experimental procedures were performed following the ethical standards of the Declaration of Helsinki and were approved by the Ethics Committee of Hiroshima University Hospital (approval no. E-588-2)

Consent to participate statement: Written informed consent was obtained from each patient for using their data in research.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conception and design: TF and KK. Development of methodology: TT, KK, and KT. Acquisition data (acquired and managed patients, provided facilities, etc.): TF, KK, KM, KT, YK, KY, HS, TH, RY, RT, KI, MN, SM, HK, KH, and AG. Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): TF, KK, KM, KT, YS, KG, and KI. Writing, review, and/or revision of the manuscript: TF, KK, KM, KT, YS, KG, KI, OK, and NH. Administrative, technical, or material support (i.e., reporting or organizing data and constructing databases): TF, KK, KM, KT, YS, KG, and KI. Study supervision: KK, OK, and NH.

Data Availability Statement

Publicly available datasets generated or analyzed during this study are included in this article. The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon reasonable request.

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Figure Legends

Fig. 1. Correlation between Nesprin1 expression and prognosis of patients with clear cell renal cell carcinoma (ccRCC). (a) Representative Nesprin1-positive and -negative ccRCC tumor tissues ($\times 200$). (b) Representative image of immunostaining to detect Nesprin1 in ccRCC, tumor capsule, and nonneoplastic kidney tissue ($\times 100$). (c) Kaplan-Meier curves of overall (upper left), cancer-specific (upper right), and recurrence-free (lower) survival rates in Nesprin1-positive and -negative nephrectomized patients with ccRCC. Hazard ratio (HR) and p values calculated by a Cox proportional hazards model and the log-rank test, respectively, are shown. The number of patients at risk (No. at risk) is investigated every 50 months for 150 months.

Fig. 2. Nesprin1 knockdown enhances invasion and migration of RCC cells. (a) The expression of Nesprin1 and β -actin in 786-O and ACHN cells 72 hours after the transfection of two different siRNAs targeting *SYNE1* (siSYNE1-1 and siSYNE1-2) and control siRNA (siCtrl) was examined by western blot analysis. The positions of molecular weight standards are shown on the left sides. (b) The resulting 786-O (left panels) and ACHN (right panels) cells were subjected to Transwell invasion assay ($n = 4$). Representative Diff-Quick stained Transwell membranes are depicted. (c) Representative images demonstrating wound-healing assay using siSYNE1 and siCtrl-treated 786-O (left panels) and ACHN (right panels) cells ($n = 6$). (d) Cell proliferation assay was performed in siSYNE1 and siCtrl-treated 786-O (left panel) and ACHN (right panel) cells; the change in the abundance of viable cells is depicted ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, determined by one-way ANOVA with Dunnett's multiple comparisons test. ns: not significant.

Fig. 3. Gene set enrichment analysis (GSEA) was performed on differential transcriptome data acquired using siSYNE1- and siCtrl-introduced 786-O and ACHN cells. (a) Venn diagram indicating the hallmark gene set pathways significantly ($p < 0.05$) induced by *SYNE1* knockdown. (b) Enrichment patterns of hallmark oxidative phosphorylation-related gene sets in 786-O (upper panes) and ACHN (lower panels) cells deduced by comparing treatments with siCtrl and siSYNE1-1 (left panels) or siSYNE1-2 (right panels); corresponding nominal (NOM) p values, false discovery rate (FDR) q values, and normalized enrichment scores (NES) are shown.

Fig. 4. Relationship between *SYNE1* expression and prognosis of sunitinib- and avelumab plus axitinib-treated patients with clear cell renal cell carcinoma (ccRCC). Kaplan-Meier curves of

progression-free survival of sunitinib (left panel)- and avelumab plus axitinib (right panel)-treated (a) overall and (b) PD-L1-positive patients are shown. *p* values calculated by the log-rank test comparing all groups are indicated. The numbers at risk (No. at risk) are indicated every 5 months, up to 25 months.