Cancer Immunology, Immunotherapy (2024) 73:231
https://doi.org/10.1007/s00262-024-03827-x

RESEARCH

A monoclonal antibody recognizing CD98 on human embryonic stem cells shows anti-tumor activity in hepatocellular carcinoma xenografts

Keunpyo Lim[1] · San Ha Han[1] · Sein Han[1] · Ji Yoon Lee[1] · Hong Seo Choi[1] · Dongho Choi[2] · Chun Jeih Ryu[1]

Received: 15 June 2024 / Accepted: 3 September 2024

Abstract

CD98, also known as SLC3A2, is a multifunctional cell surface molecule consisting of amino acid transporters. CD98 is ubiquitously expressed in many types of tissues, but expressed at higher levels in cancerous tissues than in normal tissues. CD98 is also upregulated in most hepatocellular carcinoma (HCC) patients; however, the function of CD98 in HCC cells has been little studied. In this study, we generated a panel of monoclonal antibodies (MAbs) against surface proteins on human embryonic stem cells (hESCs). NPB15, one of the MAbs, bound to hESCs and various cancer cells, including HCC cells and non-small cell lung carcinoma (NSCLC) cells, but not to peripheral blood mononuclear cells (PBMCs) and primary hepatocytes. Immunoprecipitation and mass spectrometry identified the target antigen of NPB15 as CD98. CD98 depletion decreased cell proliferation, clonogenic survival, and migration and induced apoptosis in HCC cells. In addition, CD98 depletion decreased the expression of cancer stem cell (CSC) markers in HCC cells. In tumorsphere cultures, the expression of CD98 interacting with NPB15 was significantly increased, as were known CSC markers. After cell sorting by NPB15, cells with high expression of CD98 (CD98-high) showed higher clonogenic survival than cells with low expression of CD98 (CD98-low) in HCC cells, suggesting CD98 as a potential CSC marker on HCC cells. The chimeric version of NPB15 was able to induce antibody-dependent cellular cytotoxicity (ADCC) against HCC cells in vitro. NPB15 injection showed antitumor activity in an HCC xenograft mouse model. The results suggest that NPB15 may be developed as a therapeutic antibody for HCC patients.

Keywords Hepatocellular carcinoma · Monoclonal antibody NPB15 · CD98 · HCC xenograft mouse model · Antitumor activity

Abbreviations

ADCC	Antibody-dependent cellular cytotoxicity
ALDH	Aldehyde dehydrogenase
CSC	Cancer stem cells
α-CD98	Rabbit anti-CD98 antibody
HCC	Hepatocellular carcinoma
HNSCC	Head and neck squamous cell carcinoma
hESC	Human embryonic stem cell
NSCLC	Non-small cell lung carcinoma
PBMC	Human peripheral blood mononuclear cell
PEI	Polyethyleneimine
PI	Propidium iodide
SA-HRP	Streptavidin–horseradish peroxidase
siCon	Control siRNA
siCD98	CD98 siRNA

✉ Chun Jeih Ryu
cjryu@sejong.ac.kr

[1] Department of Integrative Bioscience and Biotechnology, Institute of Bioscience, Sejong University, Seoul 05006, Republic of Korea

[2] Department of Surgery, Hanyang University College of Medicine, Seoul 04763, Republic of Korea

Introduction

CD98 (SLC3A2, 4F2) is a solute carrier family protein that is a component of the amino acid transporter and pairs with another solute carrier family protein, such as L-type amino acid transporter 1 (LAT1/SLC7A5) or cystine/glutamate transporter (xCT/SLC7A11) [1]. CD98 was discovered by biochemically analyzing the antigen bound by 4F2 monoclonal antibody (MAb) that binds to peripheral blood monocytes and activated lymphocytes [2, 3]. CD98 is known to play a role in delivering amino acids into and out of cells as an amino acid transporter. In particular, it pairs with LAT1 to deliver branched chain amino acids and aromatic amino

Published online: 11 September 2024

acids into cells, playing an important role in maintaining the cell cycle and nucleotide pool [4]. Another function of CD98 is to pair with xCT to deliver cystine into cells. Cystine is a material that synthesizes intracellular glutathione and plays an important role in protecting cells by reducing intracellular active oxygen and maintaining redox balance [5]. CD98 overexpression is closely correlated with poor prognosis in advanced tumors and overall survival in various cancers including lung and liver cancer [6–9]. Recent studies have also reported that CD98-positive cells exhibit cancer stem cell (CSC)-like properties in all head and neck squamous cell carcinoma cell lines [10, 11]. Therefore, the CD98-targeting MAb was shown to be effective in treating acute myeloid leukemia [12]. Although high level expression of CD98 is also observed in most hepatocellular carcinoma (HCC) patients [9], the role of CD98 in HCC cells has been little studied.

By injecting naïve human embryonic stem cells (hESCs) into mice, in a previous study, we generated a panel of MAb that bound to naïve hESCs [13]. Among the MAbs, NPB15 was able to bind to both naïve and primed hESCs. In addition, NPB15 antigen was not expressed in normal cells, such as peripheral blood mononuclear cells (PBMCs) and hepatocytes, but was highly expressed in HCC cells, embryonal carcinoma cells and non-small cell lung carcinoma (NSCLC) cells. Immunoprecipitation and mass spectrometry identified the NPB15 antigen as CD98. Based on the previous report [14], we postulated that NPB15 is a novel antibody that recognizes the oncofetal epitope of CD98 expressed on hESCs and HCC cells but not on normal primary cells. In this study, we report that CD98 promotes cell proliferation, clonogenic survival, migration, apoptosis resistance and cancer stemness in HCC cells. In addition, we revealed that NPB15 injection has antitumor activity in a xenograft mouse model of HCC. The results suggest that NPB15 is a novel antibody that can inhibit cancer growth by targeting CD98 on HCC cells.

Materials and methods

Cell culture

Rimed H9 hESC line (WiCell, Madison, WI, USA) was purchased and cultured on irradiated mouse embryonic fibroblasts (MEFs) as described previously [13]. To induce the naïve state of H9 hESCs, H9 hESCs were plated as small clumps on fresh inactivated MEFs in 2i/L/F/A or 2i/L/X/F/P naïve conversion media [13]. 2i/L/F/A medium consisted of KO-DMEM (Thermo Fisher Scientific) supplemented with 20% KOSR, 0.1 mM βmercaptoethanol, 12 ng/ml bFGF, 1000U recombinant human LIF (rhLIF, PrimeGene, Shanghai, China), 1 μM PD0325901 (Sigma-Aldrich), 3 μM CHIR99021 (Tocris, Seongnam, Korea), 10 μM Forskolin (Sigma-Aldrich), 50 ng/ml ascorbic acid (Sigma-Aldrich). 2i/L/X/F/P medium consisted of DMEM/F12 (Thermo Fisher Scientific) supplemented with 20% KOSR, 0.1 mM β-mercaptoethanol, 10 ng/ml bFGF, 20 ng/ml rhLIF, 1 μM PD0325901, 3 μM CHIR99021, 4 μM XAV939 (Sigma-Aldrich), 10 μM Forskolin (Tocris) and 2 μM Purmorphamine (Tocris). NT2/D1 (ATCC, Manassas, VA, USA) and HEK293FT cells (Themo Fisher Scientific, Waltham, MA, USA) were cultured in DMEM (Welgene, Gyeongsan, Korea) supplemented with 2 mM L-glutamine (Welgene), 10% fetal bovine serum (FBS)(VWR, Radnor, PA, USA) and penicillin–streptomycin solution (Welgene). 2102Ep cells were kindly provided by Professor Kim Kye-Seong of Hanyang University (Seoul, Korea). Cells were subcultured every 3 days and early passages (passage 6–8) were used in this study. Human HCC cell lines (SNU387, SNU449, Huh7, HepG2, PLC/PRF/5), a mouse HCC cell line (Hepa 1–6) and NSCLC cell lines (A549, NCI-H460, Calu-3) were purchased from the KCLB (Seoul, Korea) and ATCC and were cultured according to the instructions provided by the supplier. Human primary hepatocytes were purchased from Thermo Fischer Scientific. Human peripheral blood mononuclear cells (PBMCs) were isolated by the Ficoll-Paque Plus method (GE Healthcare, Seoul, Korea). Differentiation of H9 hESCs was induced by incorporating all trans-retinoic acid (RA, Sigma-Aldrich, St. Louis, MO, USA) at 10^{-5} M into the medium and culturing the cells for at least 7 days. Tumorsphere cultures were performed as described previously [15]. Briefly, cells were seeded at 2.1×10^3 cell/cm^2 in ultra-low attachment plate (Corning, Seoul, Korea) and maintained in DMEM/F12 (Corning) supplemented with 20 ng/ml fibroblast growth factor 2 (R&D system, Seoul, Korea), 20 ng/ml epidermal growth factor (PeproTech, Seoul, Korea) and 1 × B27 supplement (Life technologies). The medium was replaced every 3 days. All experiments were performed with mycoplasma-free cells.

Generation and purification of MAb NPB15

NPB15 was purified from the culture supernatant of hybridoma NPB15 by protein G-agarose column chromatography [13].

Flow cytometry and cell sorting

Flow cytometry was performed as described previously [13, 16]. Primary antibodies used were NPB15, Chi-NPB15, anti-human CD98 (Ancell corporation, Bayport, MN, USA), anti-mouse CD98 (BioLegend, San Diego, CA, USA), anti-CD133 (Abcam, Cambridge, UK), anti-EpCAM (Santa Cruz Biotechnology, Dallas, TX, USA) and anti-CD44 (Invitrogen, Waltham, MA, USA). For cell sorting, cancer cells (1.5

$\times 10^7$) were subjected to sorting by the expression level of CD98. Cells were labeled with 3 µg of NPB15 per 3×10^5 cells followed by staining with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Thermo Fischer Scientific). To isolate live cells, the cells were stained with propidium iodide (PI), and PI-negative cells were gated. In the cancer cell group to which NPB15 was bound, the upper 30% and lower 30% cell groups were gated and sorted on the BD FACSAria II (BD Biosciences, San Jose, CA, USA).

Cell surface biotinylation, immunoprecipitation and western blot

Cell surface biotinylation, immunoprecipitation, and Western blotting were performed as described previously [13, 16]. Briefly, biotin-labeled cells were solubilized with lysis buffer (25 mM Tris–HCl, pH7.5, 250 mM NaCl, 5 mM EDTA, 1% nonidet P-40) containing protease inhibitors (2 µg/ml aprotinin, 100 µg/ml PMSF, 5 µg/ml leupeptin, 1 mM NaF and 1 mM $NaVO_3$) at 4 °C for 30 min. Immunoprecipitation and Western blotting were performed as described previously [13]. Biotin-labeled lysates were immunoprecipitated and analyzed by Western blotting with streptavidin–horseradish peroxidase (SA-HRP). The primary antibodies used were as follows: anti-DDDDK (MBL biological, Tokyo, Japan), anti-CD98 (Cell signaling technology, Danvers, MA, USA), anti-EpCAM (Abcam, Cambridge, UK), anti-CD13 (Cell signaling technology) and anti-α-tubulin antibodies (Proteintech, Rosemont, IL, USA). For western blot on formaldehyde-treated tumor tissues, tumors were first homogenized with a mortar and pestle, and proteins were extracted overnight on a rotator at 4 °C with RIPA buffer (150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris–HCl (pH 7.4), 1% NP40, 2 µg/ml aprotinin, 100 µg/ml PMSF, 5 µg/ml leupeptin, 1 mM NaF, 1 mM Na_3VO_4). The signal intensities of Western blots were measured quantitatively using the Image J software.

Mass spectrometry

The protein of interest was enzymatically digested in-gel in a manner similar to that described previously [13].

Wound healing assay

Cells (1.5×10^5) were seeded into 24 plates per well and incubated overnight to make cells reach 90% confluence. The day after seeding, the cell monolayer was scratched using yellow pipette tips. To remove the cell debris, the culture media was aspirated and washed with PBS (pH 7.4) twice. The media was replaced with 5% FBS and incubated at 37 °C for 24 h. The scratch was photographed at 0 and 12 h after scratch, and the scratch area was quantified by Image J software.

Transfection

To perform CD98 knockdown in HCC cells, HCC cells were transfected with negative control siRNA (Genolution, Seoul, Korea), CD98 siRNA#1 (siCD98, Bioneer#6520–1, Daejeon, Korea) and CD98 siRNA#2 (10). Briefly, cells (5×10^4) were seeded on 6-well plates with antibiotics-free media. The day after seeding, transfection was performed using siRNAs with RNAiMAX (Thermo Fischer Scientific) following the manufacturer's protocol. The medium was changed 8 h after transfection, and the cells were harvested 72 h after transfection. For ectopic expression of Flag-tagged CD98, HEK293FT cells were seeded the day before transfection. pCMV3-SLC3A2-FLAG expression plasmid (Sino Biological, Beijing, China) and polyethyleneimine (PEI, Polyscience, Warrington, PA, USA) were mixed in the TOM medium (Welgene) at a ratio of 1:3. The transfected HEK293FT cells were harvested after 72 h after transfection and used for immunoprecipitation.

Clonogenic survival and apoptosis assay

Cancer cells knocked down by CD98 siRNA or sorted according to CD98 expression level were recovered and were subjected to clonogenic survival assay as described previously [17]. Briefly, siCon or siCD98 transfected cells were harvested at 48 h after siRNA transfection. The cells (1×10^3) were plated in a 6-well plate with complete medium and incubated for 10-day incubation, the cells were stained with crystal violet (Sigma-Aldrich) at room temperature for 1 h, and the colonies were counted. Detection of apoptosis was measured by staining with PI and FITC-conjugated annexin V kit (BD Biosciences). The assay was performed following the manufacturer's protocol.

Construction, expression, purification and analysis of chimeric NPB15 antibody

Total RNAs were isolated from hybridoma NPB15 cells by using RNAiso Plus (TaKaRa, Otsu, Japan), and cDNA synthesis was performed by using PrimeScript™ RT Master Mix (TaKaRa). All the experiments followed the manufacturer's instructions. To clone the cDNAs coding the NPB15 antibody variable domain, primers were prepared as described previously [18]. To produce the chimeric antibody version of NPB15, the variable regions of heavy and kappa chain were fused with human heavy/light chain signal peptide DNA fragments by recombinant PCR using eight synthetic oligonucleotides as primers (Table S1). The fused products of heavy chain were digested with EcoRI

and ApaI (Enzynomcis, Daejeon, Korea) and inserted into the front of the human Cγ1 sequence to generate pdCMV-dhfr-Chi-NPB15 expression vector. In the same manner, the fused fragments of light chain were digested with HindIII and BsiWI (Enzynomcis) and inserted into the front of Cκ sequence of pdCMV-dhfr-Chi-NPB15 expression vector [19]. The expression plasmids were transiently expressed in HEKMAb cells by transfection using polyethyleneimine. The chimeric NPB15 antibody was purified from the culture supernatant by affinity column chromatography with recombinant protein G-agarose (Amicogen, Seoul, Korea).

ALDH activity assay

ALDH (aldehyde dehydrogenase) activity assay was performed by using ALDH Activity Assay Kit (Abcam, Cambridge, UK) according to manufacturer's protocol. Briefly, Huh7 and SNU449 cells (1.0×10^6) were homogenized with 200 µl icy cold assay buffer and incubated at 4 °C for 10 min. Cells were centrifuged, and supernatants were transferred to 96-well plates and subjected to be samples for test. The reaction mixture containing developer/substrate, reaction buffer and acetaldehyde was added and incubated at room temperature in the dark. ALDH activity was measured at OD450 nm at 5 and 25 min after incubation, and the relative ALDH activity was calculated as follows: Relative ALDH activity = OD450nm at 25 min – OD450nm at 5 min incubation.

ADCC reporter gene assay

Huh7 cells (7.5×10^4) cells were plated on 96-well white-sided microtiter plate. The antibody-dependent cellular cytotoxicity (ADCC) reporter assay was performed in accordance with the manufacturer's instructions (Promega, Madison, WI, USA). The chimeric NPB15 antibody was added to Huh7 cells with ADCC bioassay effector Jurkat cells at 25:1 E/T ratio. Six hours after co-culture at 37 °C in the presence of the chimeric NPB15 antibody, luciferase assay reagent was added into each well. The luminescence was detected by luminometer (Promega).

Xenograft nude mouse model of HCC and in vivo *antitumor activity of NPB15*

Four- to five-week-old female immune-deficient nude (BALB/c nu/nu) mice were purchased from Nara biotech (Seoul, Korea). To establish HCC xenograft mouse models, 5×10^6 Huh7 cells were resuspended in 200 µl of a 1:1 mixture of Matrigel (Thermo Fischer Scientific) and PBS and injected into the dorsal right flank of 12 nude mice subcutaneously. Tumor growth in mice was assessed by measuring the tumor size twice per week using calipers (length/2 × width × width). When tumor volume reached to 100 mm^3, 10 mg/kg of NPB15 ($n = 6$) or isotype mouse IgG1 ($n = 6$) was intravenously or intraperitoneally injected into the mice every 2 days. All mice were killed when tumor volumes reached about 2000 mm^3. Tumor volume and the weight of the mice were measured every 2 days. All animal experiments were approved by the Institutional Animal Care and Use Committee at Sejong University (SJ-20220512).

Statistical analyses

Statistical evaluation for data analysis was determined by unpaired Student's t test, one-way and two-way ANOVA. All experiments were performed at least three times. All data are presented as mean values ± SD except for tumor-related data. Tumor width and length were determined using a caliper. Data were presented as mean values ± SEM for isotype ($n = 6$) and NPB15 ($n = 6$). A p value < 0.05 is indicated by *, $p < 0.01$ by ** and $p < 0.001$ by ***.

Results

NPB15 binds to hESCs, HCC and NSCLC cells but not to PBMC and hepatocytes

Previously, we generated a panel of MAbs specific to naïve hESCs by using primed hESCs as a decoy immunogen [13]. In the same mice, primed hESCs were first injected into the right foot as a decoy immunogen, and naïve hESCs were injected into the left foot as a target immunogen, and then the left popliteal lymph nodes were collected to produce hybridomas. Among the hybridomas produced in this way, many hybridomas produced antibodies that simultaneously bound to primed and naïve hESCs [13]. NPB15, one of the MAbs, bound to naive and primed hESC, but not to mouse embryonic fibroblasts in flow cytometric analysis (Fig. 1a). When primed hESCs were induced to differentiation by retinoic acid, NPB15 binding was drastically decreased (Fig. 1a). NPB15 also bound to various cancer cells, including embryonic carcinoma cell lines (NT2/D1 and 2102Ep), human HCC cell lines (Huh7, HepG2, SNU387, SNU449, PLC/PRF/5, SK-Hep1) and human NSCLC lines (NCI-H460, A549, Calu-3), but did not bind to normal PBMC and human primary hepatocyte (Fig. 1b, supplementary Table S1). The results suggest that NPB15-reactive antigen is a cell surface molecule that is expressed in naïve and primed hESCs but is significantly reduced in differentiated cells. The results also suggest that NPB15-reactive antigen is expressed in HCC and NSCLC cells, but not in PBMCs and hepatocytes.

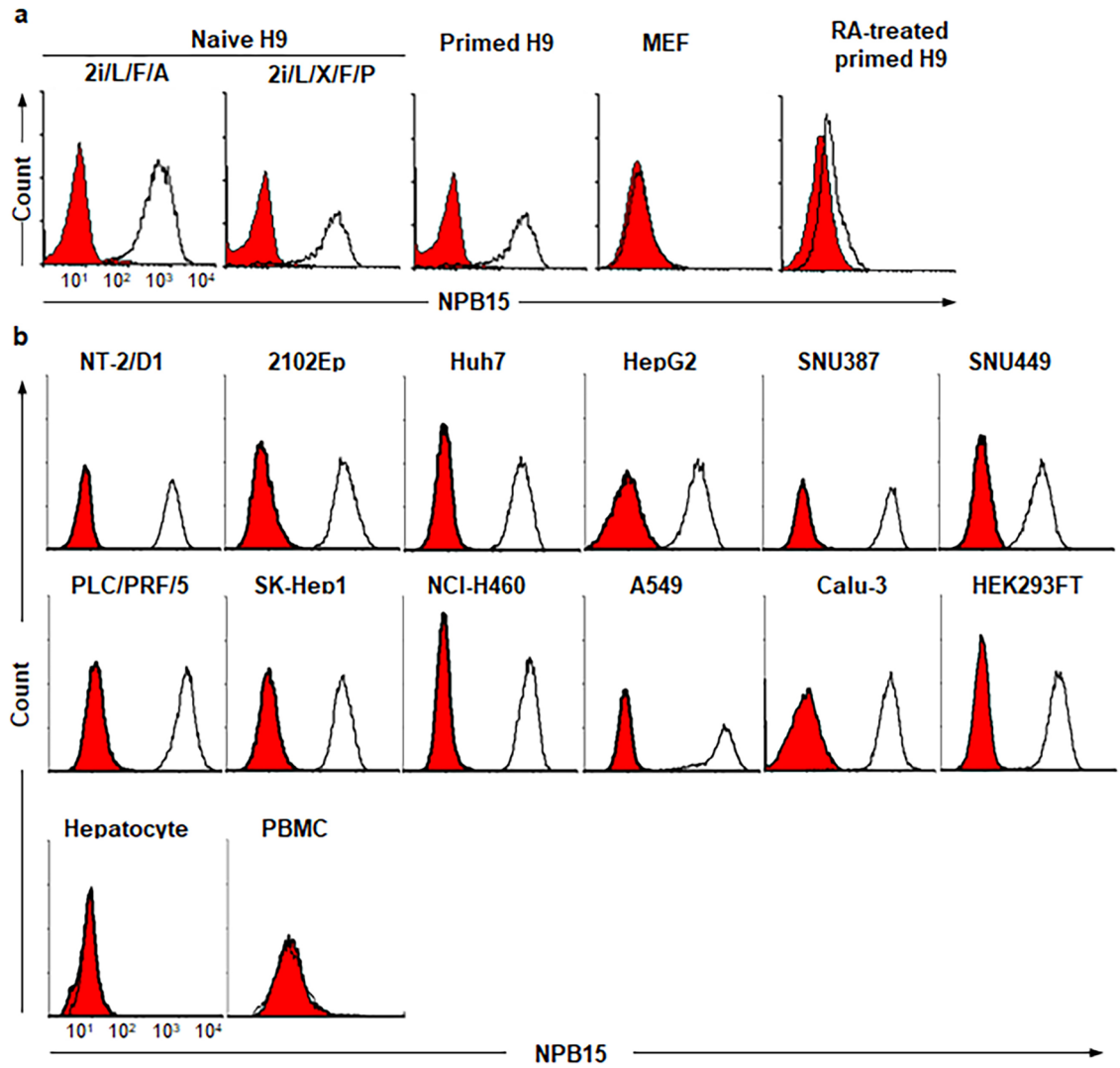

Fig. 1 Flow cytometric analysis of various cells with NPB15. **a** Binding profiles of NPB15 were examined by flow cytometry in naïve and primed hESCs, mouse embryonic fibroblasts (MEFs) and retinoic acid-treated primed H9 cells. **b** Binding profiles of NPB15 were examined by flow cytometry in various cancer cell lines and PBMCs and hepatocytes

NPB15 recognizes the extracellular domain of CD98 in a conformation-dependent manner

NPB15 appeared to recognize proteins expressed on the surface of hESCs, HCC cells and NSCLC cells rather than normal cells. To identify the target antigen recognized by NPB15, surface proteins of NT2/D1 and A549 cells were biotinylated, cell surface proteins were immunoprecipitated with NPB15, and the immunoprecipitants were analyzed by Western blotting with SA-HRP. NPB15 immunoprecipitated approximately 90 and 120 kDa surface proteins on NT2/D1 and A549 cells (Fig. 2a, supplementary Fig. S1a). However, the NPB15-reactive proteins were not detected in the NT2/D1 whole cell lysates by Western blotting with NPB15 (Supplementary Fig. S1b), suggesting that NPB15 recognizes its cognate antigen in a conformation-dependent manner. To identify the antigen, the NPB15 immunoprecipitants were stained with PageBlue solution, the 120 kDa band was excised, and the excised band was subjected to mass spectrometry (Supplementary Fig. S1c). The 120 kDa protein was identified as CD98 from a protein database search (Supplementary Fig. S1d). To prove whether NPB15 recognizes CD98, A549 cell lysates were immunoprecipitated with NPB15, and the immunoprecipitated proteins were detected with a commercially available rabbit anti-CD98 antibody (α-CD98). The immunoprecipitant was detected at approximately 90 and 120 kDa by Western blotting with α-CD98, suggesting that NPB15 immunoprecipitates CD98 (Fig. 2b). To further prove whether CD98 is the target antigen of NPB15, HEK293FT cells were transfected with

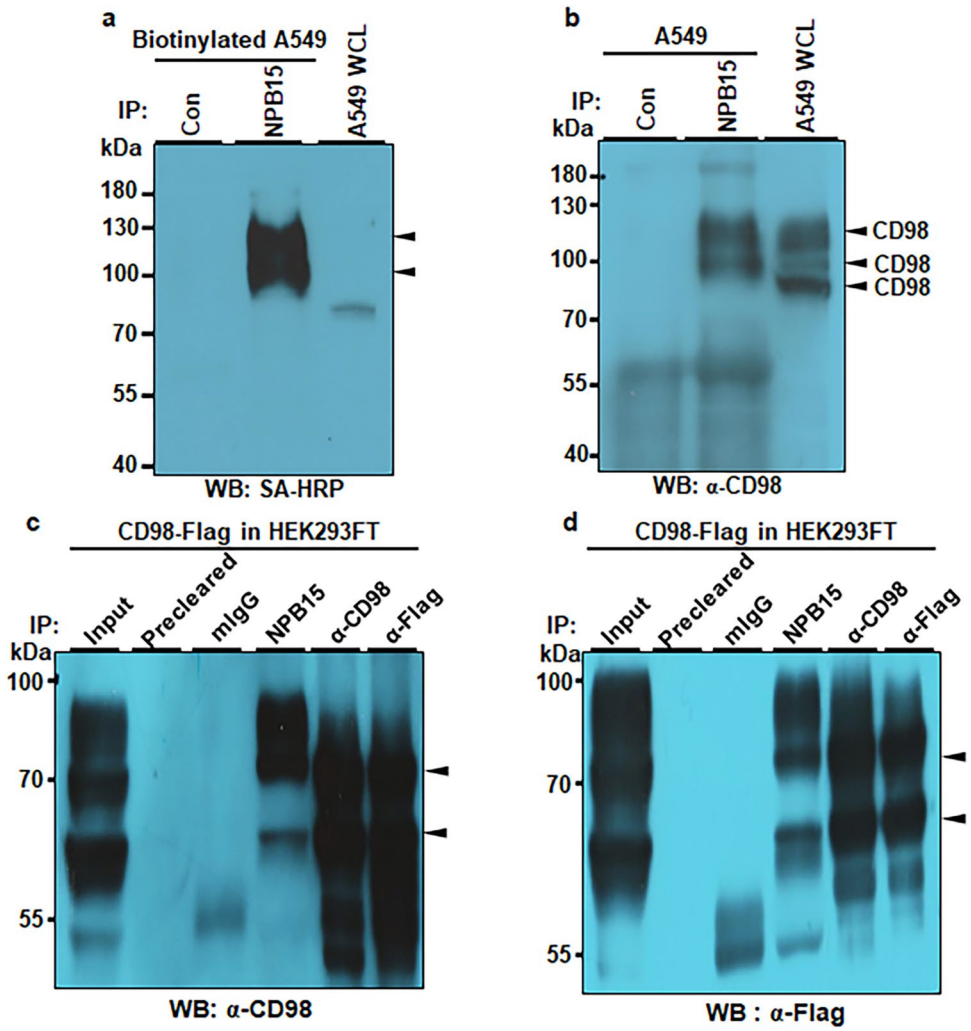

Fig. 2 Identification and validation of cell surface molecule recognized by NPB15. **a** A549 cell lysates were immunoprecipitated with NPB15 after cell surface biotinylation, and the immunoprecipitants were detected with SA-HRP in Western blotting. Preclearing was done with protein G-agarose beads alone and used as a control (Con). **b** A549 cell lysates were immunoprecipitated with NPB15, and the immunoprecipitants were detected with α-CD98 in Western blotting. **c**, **d** Immunoprecipitation of FLA-tagged CD98 with mouse IgG (mIgG), NPB15, α-CD98 and α-FLAG antibodies after overexpression of FLAG-tagged CD98 in HEK293FT cells. Immunoprecipitants were analyzed by Western blotting with α-CD98 (**c**) or α-FLAG antibodies (**d**). Preclearing was done with protein G-agarose beads alone and used as a control

control and pCMV3-SLC3A2-FLAG expression vectors, and the cell lysates were subjected to immunoprecipitation with NPB15, α-CD98 and anti-FLAG, and then detected with α-CD98 or α-FLAG. Two major recombinant CD98 proteins were detected in all three immunoprecipitants with α-CD98 and or α-FLAG (Fig. 2c, d). Taken together, the results suggest that NPB15 directly recognizes extracellular domain of CD98 in a conformation-dependent manner.

CD98 depletion inhibits cell proliferation and promotes apoptosis

To assess loss of function of CD98, cancer cells were knocked down by transfection with two CD98 siRNAs or negative control siRNA (siCon). Among the two CD98 siRNAs, one (siCD982) showed a knockdown efficiency of approximately 33%, but the other (siCD98) showed more than 70% knockdown efficiency in all cells used, so siCD98 was used in all subsequent experiments (supplementary Fig. S2). Western blot analysis showed that CD98 protein expression was downregulated up to approximately 99% and 72% by siCD98 transfection in SNU449 and Huh7 cells, respectively (Fig. 3a). Thus, siCD98 induced more than 70% reduction in CD98 protein in both HCC cells. Cell surface expression of CD98 was also decreased in siCD98 SNU449 and Huh7 cells (Fig. 3b). CD98 depletion decreased the number of SNU449 and Huh7 cells by approximately 31% and 34%, respectively, as compared to those of siCon group (Fig. 3c). The results suggest that CD98 is required for cell proliferation of HCC cells. To investigate whether CD98 plays a role in apoptosis resistance, CD98-depleted cells were stained with annexin V and PI. Although single annexin V-positive cells were not changed in siCD98 SNU449 cells, both annexin V- and PI-positive cells were increased by 15.4% in siCD98 SNU449 cells (Fig. 3d, f). Both annexin V- and PI-positive cells were not changed in siCD98 Huh7 cells, but single annexin V-positive cells were increased by 34.6% in siCD98 Huh7 cells (Fig. 3e, g), suggesting that CD98 is required for apoptosis

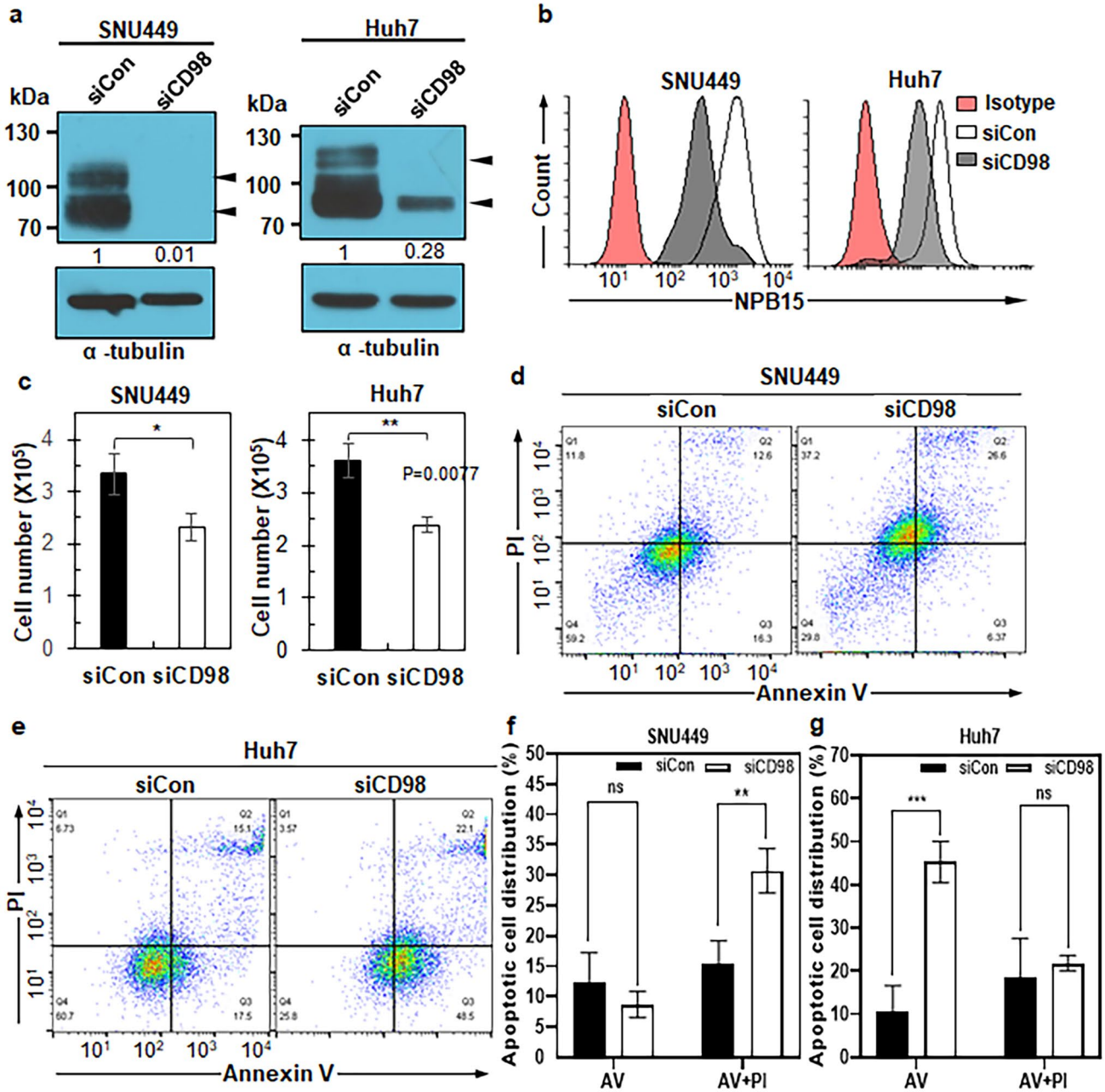

Fig. 3 CD98 depletion inhibits cell proliferation by promoting apoptosis in HCC cells. **a** CD98 protein expression of the control (siCon) and CD98 knockdown (siCD98) SNU449 and Huh7 cells. Indicated protein levels were analyzed by Western blotting and Image J software. **b** Flow cytometry of the CD98 expression in siCon and siCD98 SNU449 and Huh7 using NPB15. **c** Quantification of cell numbers of siCon and siCD98 SNU449 and Huh7 cells (*$p < .05$; ***$p < .005$). **d, e** Apoptosis analysis of siCon and siCD98 SNU449 and Huh7 cells. Cells were stained with PI and annexin V. **f, g** Quantification of **d** and **e** (ns, not significant; **$p < .01$; ***$p < .005$)

resistance in HCC cells. Thus, CD98 is required for the promotion of cell proliferation and the suppression of apoptosis in HCC cells.

CD98 depletion inhibits clonogenic survival and migration

To investigate the role of CD98 in clonogenic survival of HCC cells, clonogenic survival assays were performed using the siCD98 SNU449 and Huh7. CD98 depletion reduced the number and size of SNU449 and Huh7

colonies by 70% and 97%, respectively (Fig. 4a, b). The results demonstrate that CD98 promotes cell survival and proliferation under low-density seeding stress. To investigate the role of CD98 in migration potential essential for metastasis of cancer cells, wound healing assays were performed using siCD98 SNU449 and Huh7 cells. After 12 h, the scratch area of the control group was reduced by approximately 34%, while the scratch area was reduced by approximately 8% in siCD98 SNU449 cells (Fig. 4c, d). Similarly, the scratch area was reduced in siCon group by approximately 24% after 12 h, while the scratch area was reduced by approximately 6% in siCD98 Huh7 cells (Fig. 4e, f). Taken together, the results demonstrate that CD98 promotes clonogenic survival and migration in HCC cells.

CD98 expression is positively associated with *cancer* stemness

CSCs are thought to be responsible for tumor growth, drug resistance and recurrence [14]. Since CD98 was closely associated with cancer growth and clonogenic survival, we investigated whether the expression level of CD98 is closely related to cancer stemness. To investigate whether CD98 depletion affects cancer stemness, cell surface expression of CD44, a CSC marker, was examined in cancer cells by flow cytometry. When cell surface expression of CD98 was decreased by 69% in siCD98 SNU449 cells, cell surface expression of CD44 was also decreased by 46% (Fig. 5a, b). Since EpCAM and CD13 are known CSC markers in Huh7 cells [20], we also examined EpCAM and CD13 protein expression in Huh7 cell. EpCAM protein was decreased by approximately 54% in siCD98 Huh7 cells, whereas CD13 protein expression was not significantly altered (Fig. 5c, d). ALDH is also considered as a classical and reliable biomarker of CSCs in HCC [20, 21]. CD98 depletion also decreased the expression levels of ALDH by approximately 19% and 18% in SNU449 and Huh7 cells, respectively (Fig. 5e). The results suggest that CD98 expression is positively associated with cancer stemness in HCC cells. Tumorsphere cultivation is a method for amplifying and enriching CSCs in vitro [22, 23]. To further examine correlation of NBP15-reactive CD98 with CSC markers on tumorsphere cells, adherent Huh7 cells were detached and cultured in tumorsphere forms for at least 9 days. Cell surface expression of liver CSC markers CD133, EpCAM and CD44 [20] was increased by 1.4-fold, 1.5-fold and 1.6-fold, respectively, in tumorsphere culture, where cell surface expression of CD98 was also increased by 1.4-fold (Fig. 5f–h). The results suggest that CD98 is positively associated with cancer stemness in HCC cells.

Cells with high expression of CD98 show higher clonogenic survival than cells with low expression

To dissect the role of CD98 in HCC cell survival and stemness, the cell population corresponding to the top 30% (cells with high expression of CD98, CD98-high) and the cell population corresponding to the bottom 30% (cells with low expression of CD98, CD98-low) were sorted from NPB15-positive cancer cells in flow cytometric analysis (Fig. 6a). CD98-high and -low cancer cells were then subjected to clonogenic survival assays (Fig. 6b, c). CD98-high SNU449 cells showed increased clonogenic survival by 1.6-fold, as compared with CD98-low SNU449 cells (Fig. 6c). The results suggest that enhanced cell surface expression of CD98 contributes to increased clonogenic survival and stemness in HCC cells.

NPB15 shows antitumor activity in vivo

Based on the above results, CD98 promoted the growth and migration of cancer cells and appeared to be a surface marker for CSCs. Therefore, NPB15 targeting CD98 was expected to have antitumor activity in vivo. Prior to in vivo experiment, we first investigated whether NPB15 had ADCC activity in vitro. To investigate the in vitro ADCC activity of NPB15, the chimeric version of NPB15 was prepared by fusing the heavy chain variable region of NPB15 to the human IgG1 constant region and the light chain variable region of NPB15 to the human kappa constant region. The chimeric antibody (Chi-NPB15) was purified from the culture supernatant of HEK293FT after transfection of the expression vector of chimeric antibody (Supplementary Fig. S3a, b). Chi-NPB15 bound to Huh7 cells as did NPB15 (Supplementary Fig. S3c). Therefore, ADCC potential of Chi-NPB15 was investigated by in vitro ADCC reporter assay using Huh7 cells and Chi-NPB15 as described in Materials and Methods. Chi-NPB15 exhibited potent ADCC activity against Huh7 cells as compared to control human IgG1 (Fig. 7a), suggesting that NPB15 may have potent in vivo antitumor activity through ADCC activity. To investigate the in vivo therapeutic effects of NPB15, we decided to examine the effects of NPB15 in a mouse tumor model. Although there is sequence similarity between human and mouse, human CD98 (hCD98, 630 aa) and mouse CD98 (mCD98, 526 aa) are quite different from each other. In flow cytometry analysis, a commercial anti-mCD98 antibody recognized mCD98 on mouse HCC cell line Hepa 1–6 cells but not hCD98 on human HCC cell line Huh7 cells while a commercial anti-hCD98 antibody recognized hCD98 on Huh7 cells but not mCD98 on Hepa 1–6 cells (supplementary Fig. S4a, b). Under this condition, NPB15 recognized hCD98 on Huh7 cells but not mCD98 on Hepa 1–6 cells. The results suggest that NPB15 does not recognize mCD98 in induced or spontaneous HCC mouse tumor models.

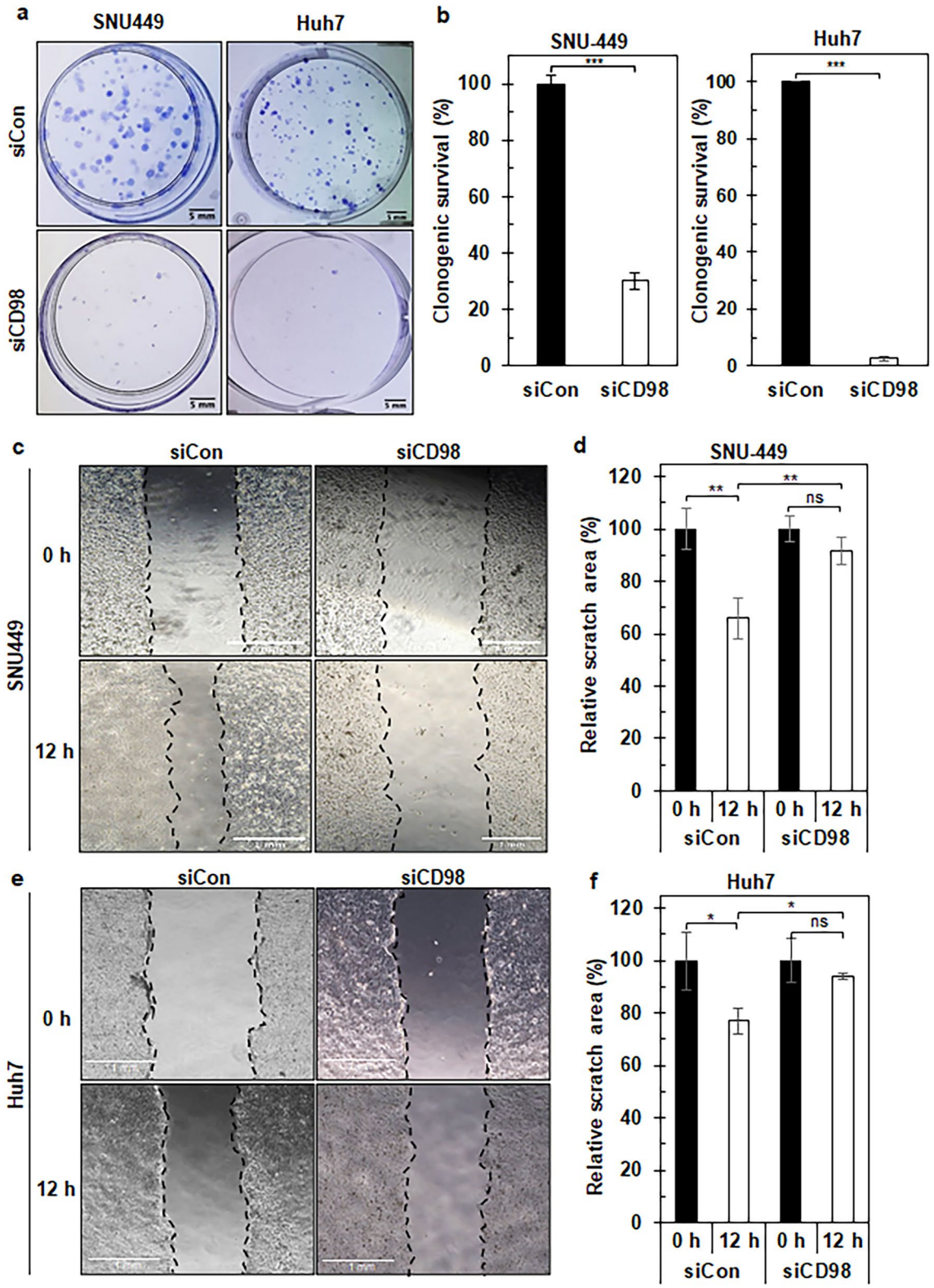

Fig. 4 CD98 depletion inhibits clonogenic survival and migration in HCC cells. **a** Clonogenic survival assays of siCD98 Huh7 and SNU449 cells. The scale bar is 5 mm. **b** Quantification of **a**. The number of colonies was quantified and normalized (clonogenic survival of siCon, 100%). **c, e** Wound healing assays with siCD98 SNU449 and Huh7 cells. The scale bar is 1 mm. **d, f** Quantification of **c** and **e**. The scratch area was measured and normalized at 0 h (*$p < .05$; **$p < .01$)

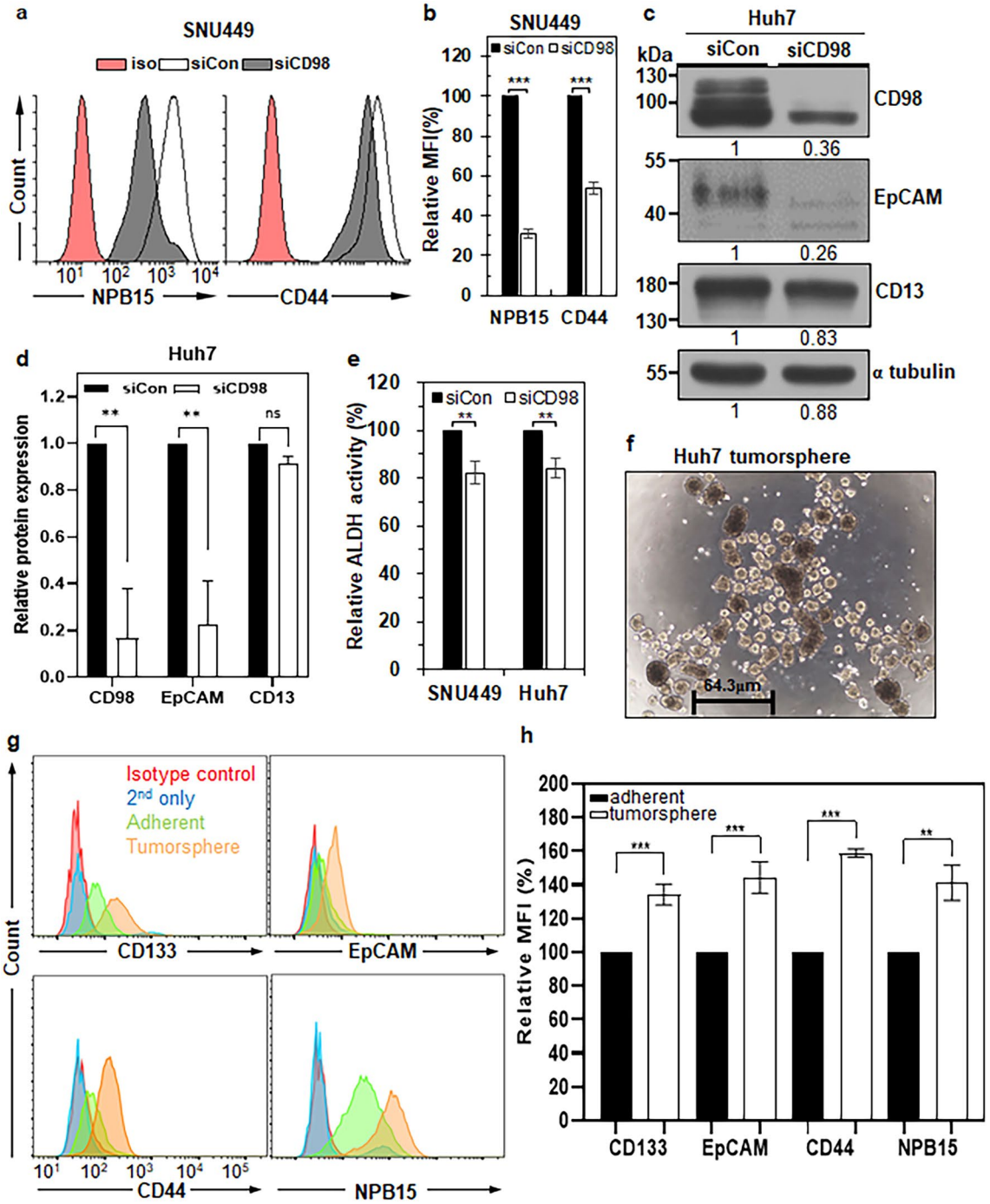

Fig. 5 CD98 expression is positively associated with cancer stemness. **a** Cell surface expression of CD44 was examined in CD98 knockdown SNU449 cells by flow cytometry. **b** Quantification of **a**. The MFIs of NPB15 and CD44 were quantified and normalized against the MFIs of control cells (siCon). **c** Protein expression of EpCAM and CD13 was analyzed in CD98 knockdown (siCD98) Huh7 cells by Western blotting. **d** Quantification of **c**. Protein expression levels of siCD98 Huh7 cells were quantified by Image J software and normalized against control cells (siCon). α-tubulin was used as a loading control. **e** ALDH activity of CD98 knockdown cancer cells. ALDH activity was measured and normalized against control cells (siCon). **f** Image of tumor spheroids (scale bar, 64.3 μm). **g, h** Flow cytometry analysis of Huh7 tumor spheroids with CSC markers. The expression levels of CD133, EpCAM, CD44 and CD98 were quantified and normalized against those of adherent cells (*$p < .05$; **$p < .01$)

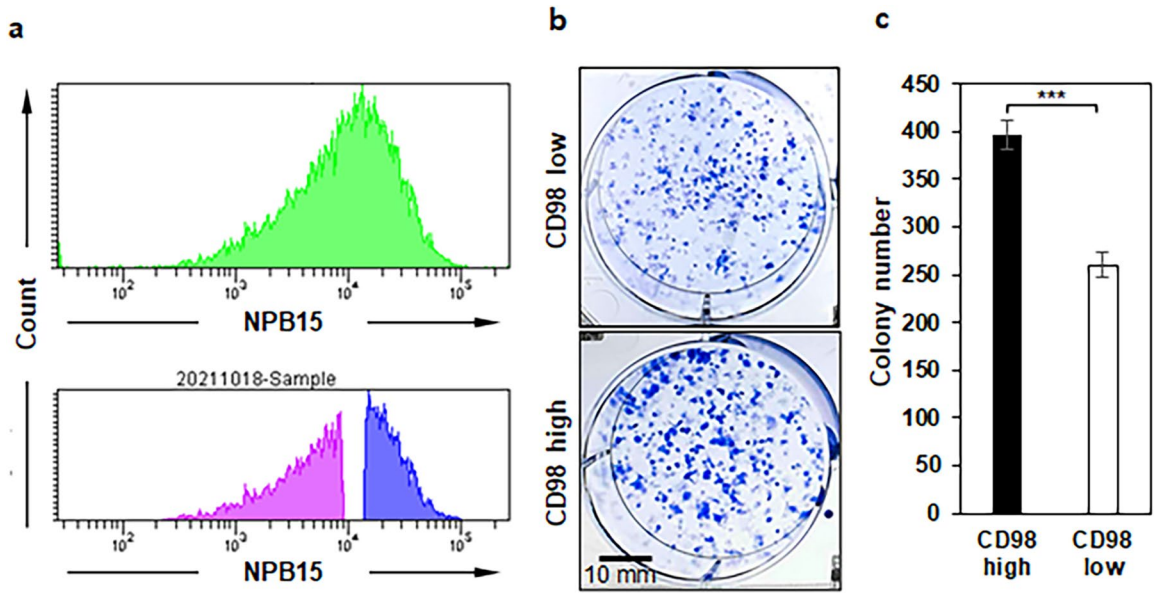

Fig. 6 NPB15-sorted CD98-high cells show higher clonogenic survival than CD98-low cells. **a** SNU449 cells were stained with NPB15, and the top 30% (CD98-high) and bottom 30% cell populations (CD98-low) were sorted on a cytometer. **b** NPB15-Sorted CD98-high and -low cells were subjected to clonogenic survival assays. The scale bar is 10 mm. **c** Quantification of b. The number of colonies was quantified by Image J software (***$p < .005$)

To investigate the therapeutic effect of NPB15 in vivo, therefore, we examined the effect of NPB15 in a xenograft nude mouse model of HCC. A group of BALB/c nu/nu mice was subcutaneously transplanted with Huh7 cells and treated with intravenous injection of control mouse IgG1 or NPB15 every 2 days after the tumor volume reached 100 mm^3 (Fig. 7b). The treatment of NPB15 resulted in sustained inhibition of growth of Huh7 tumor xenografts over the course of the 20-day study, as compared to mouse IgG1 (Fig. 7c, d). The tumor weight of the NPB15-treated group was also significantly decreased compared with the control group (Fig. 7e), and any abnormalities in both the body weight and behavior of the mice were not observed (Fig. 7f). When the extracted tumor tissues were analyzed by Western blots, NPB15 treatment significantly reduced CD98 protein expression in NPB15-treated tumor tissues compared with isotype-treated tumor tissue (Fig. 7g). The results suggest that NPB15 exhibits antitumor activity by inhibiting CD98 expression. Taken together, the results suggest that NPB15 exhibits antitumor activity in vivo and also suggest that NPB15 may be developed as a therapeutic antibody in HCC patients.

Discussion

Since cancer-associated antigens are generally expressed in normal tissues, it is not easy to discover antigens or antibodies with high specificity to cancer cells. CD98 is highly upregulated in various cancer cells and promotes cancer cell survival, migration, invasion, angiogenesis and drug resistance [24]. However, CD98 is also widely expressed in normal tissues. CD98 is also highly upregulated in patients with HCC, but basal expression level of CD98 is also observed in normal liver tissues [9]. Therefore, we expected that CD98 expression would be observed in normal hepatocytes. However, interestingly, NPB15 did not bind to normal hepatocytes and normal PBMC at all (Fig. 1B). The result is reminiscent of R8H283 MAb that binds to tumor-restricted epitope on CD98 expressed in multiple myeloma (MM) patients but not in normal lymphocytes or leukocytes [25]. Generally, CD98 is regarded as a lymphocyte activation antigen and is expressed on peripheral monocytes and activated lymphocytes [3]. However, R8H283 specifically targets CD98 on MM cells and exerts anti-MM effects without damaging normal hematopoietic cells [25]. The result indicates that MAbs targeting tumor-restricted epitopes show a marked lack of side effects and is promising antibodies for the development of antibody therapeutics. To generate and establish R8H283, the authors screened more than 10,000 MAb clones raised against MM cells. Unlike traditional CD98-targeting approaches, in this study, we generated a MAb targeting CD98 on hESCs. Embryonic stem cells and cancer cells have many biological similarities and share many markers and characteristics [26]. We postulated that the antigens recognized by hESC-specific antibodies are antigens with the characteristics of oncofetal antigen, which are expressed in embryonic and cancer cells but not in normal primary cells [14]. The basic similarities between

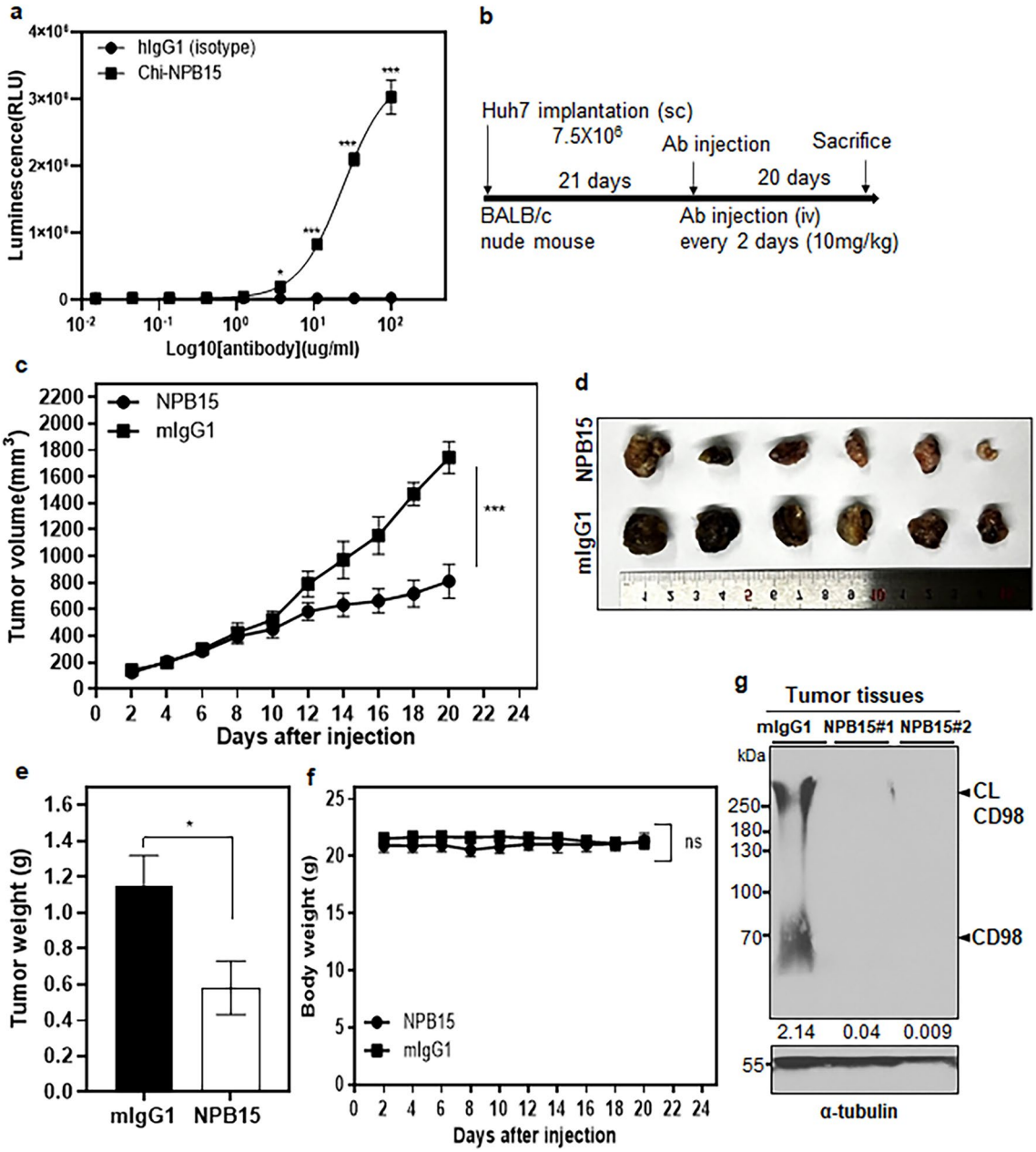

Fig. 7 NPB15 shows antitumor activity in an HCC xenograft animal model. **a** ADCC assay of chimeric NPB15 antibody on Huh7 cells. Huh7 cells were treated with 0 to 100 μg/ml Chi-NPB15 or human IgG1 isotype control and incubated with effector Jurkat cell for 6 h. The relative luminescence unit (RLU) is plotted against the logarithmic antibody concentration (*$p<.05$; ***$p<.005$). **b** A schematic diagram showing the workflow of antibody injection into Huh7 xenograft mice. BALB/c nu/nu mice were injected subcutaneously with Huh7 cells and injected intravenously every 2 days with mIgG1 or NPB15 after the tumor volume reached 100 mm^3. **c** Graphical representation of mIGg1- and NPB15-treated tumor growth, respectively. Tumor width and length were determined using a caliper. Data are presented as mean values ± SEM for isotype ($n=6$) and NPB15 ($n=6$) (*$p<.05$; ***$p<.005$). **d** Tumors from killed xenograft models are shown. **e** Tumors extracted (shown in **d**) were weighted and mean tumor weights ± SEM are presented (*$p<0.05$). **f** Effects of antibody injection on body weight of HCC xenograft mice. Body weights of 12 mice were measured every 2 days and mean body weights ± SEM are presented (ns, not significant). **g** Western blot analysis of CD98 in NPB15- or mIgG1-treated tumor tissues. Formaldehyde-treated tumor tissues were homogenized and analyzed by Western blot analysis with anti-CD98 antibody. α-tubulin was used as an internal loading control. CL CD98, cross-linked CD98 by HCHO

hESCs and CSCs are that both have pluripotency or multipotency, and express the same oncofetal antigens [27]. In fact, studies including our studies have discovered oncofetal antigens using hESC-specific MAbs, and the MAbs have been developed as antibodies for cancer treatment [28, 29]. NPB15, one of the MAbs, had specificity for undifferentiated hESCs and bound to various HCC cells but not to primary hepatocytes (Fig. 1). Subsequent studies demonstrated that NPB15 recognizes a tumor-specific conformational epitope on CD98 and inhibits tumor growth in an HCC xenograft mouse model. Therefore, the present study suggests that hESC-specific MAbs are a good source for discovering antibodies that recognize tumor-restricted epitopes and also for discovering antibodies for cancer treatment.

The present study showed that NPB15 recognized CD98 protein in a conformation-dependent manner and was unable to recognize CD98 protein denatured by sodium dodecyl sulfate (Supplementary Fig. S1d). The results suggest that the epitope of NPB15 is a three-dimensional conformation epitope formed on CD98 in HCC cells, and the epitope is likely to be a specific epitope for HCC cells that is not formed in normal hepatocytes and hematopoietic cells. These results were reminiscent of the antibody used to target cancer-specific CD98 in triple negative breast cancers and colorectal cancer [30, 31]. Although the authors selected CD98 among the molecules whose expression increased in cancer tissues compared to normal tissues through extensive surfaceome analyses, they further selected the antibody that recognized a conformational epitope on CD98 among antibodies made by immunizing a human leukemia cell line. Despite the superiority of CD98 protein as an anticancer target, CD98 expression is widely distributed not only in cancers but also in various cells and tissues [32, 33]. Therefore, antibody therapeutics that recognize CD98 on the surface of normal cells may cause various immune side effects. Cancer-specific antigens have generally been discovered through proteomics and transcriptomics comparisons between normal and cancerous tissues. However, cancer-specific epitopes formed by protein posttranslational modifications, such as glycosylation [34], or conformational changes [35] may be missed by proteomics or transcriptomics approaches. As shown in the present study, it can be also discovered through generation of hESC-specific MAbs. Based on the present study, NPB15 is expected to have no side effects on at least normal hepatocytes and hematopoietic cells. To develop NPB15 as an antibody therapeutic, it must be analyzed for nonspecific binding through extensive immunohistochemistry on various human normal tissues in future studies.

In summary, we generated a MAb called NPB15 by immunizing hESCs into mice. NPB15 recognized CD98 on HCC cells but did not show the binding activity to CD98 on hepatocytes and PBMCs. CD98 depletion inhibited cell proliferation, survival, apoptosis resistance, migration and cancer stemness in HCC and NSCLC cells. NPB15-sorted CD98-high cells showed higher clonogenic survival than CD98-low cells in HCC cells. NPB15 showed antitumor activity in an HCC xenograft mouse model. NPB15 is a potential candidate that can be developed as antibody therapeutics for cancer treatment in HCC patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00262-024-03827-x.

Acknowledgements We thank Dr. Hee Chul Lee for his comments and proofreading of the manuscript.

Author Contributions K. Lim was involved in development of methodology, acquisition of data, analysis and interpretation of data; S.H. Han helped in acquisition of data, development of methodology; S. Han helped in acquisition of data; J.Y. Lee helped in acquisition of data; H.S. Choi helped in acquisition of data; D. Choi contributed to resources, supervision; C.J. Ryu assisted in conception and design, analysis and interpretation of data, writing, review, and/or revision of the manuscript. All authors reviewed and approved the final manuscript.

Funding This study was supported in part by the National Research Foundation of Korea (2022R1A2C1010132 and 2016M3A9C6918220). This research was supported by the Korean Fund for Regenerative Medicine funded by Ministry of Science and ICT, and Ministry of Health and Welfare (21A0401L1).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors declare no competing interests.

Ethical approval The animal study was reviewed and approved by the Institutional Animal Care and Use Committee at Sejong University (approval number: SJ-20200304) of the Republic of Korea.

Consent to participate Not applicable.

Consent of publish Not applicable.

References

1. Lee Y, Wiriyasermkul P, Jin C et al (2019) Cryo-EM structure of the human L-type amino acid transporter 1 in complex with glycoprotein CD98hc. Nat Struct Mol Biol 26:510–517. https://doi.org/10.1038/s41594-019-0237-7
2. Eisenbarth GS, Haynes BF, Schroer JA, Fauci AS (1980) Production of monoclonal antibodies reacting with peripheral blood mononuclear cell surface differentiation antigens. J Immunol 124:1237–1244
3. Haynes BF, Hemler ME, Mann DL, Eisenbarth GS, Shelhamer J, Mostowski HS, Thomas CA, Strominger JL, Fauci AS (1981) Characterization of a monoclonal antibody (4F2) that binds to human monocytes and to a subset of activated lymphocytes. J Immunol 126:1409–1414
4. Cano-Crespo S, Chillarón J, Junza A et al (2019) CD98hc (SLC3A2) sustains amino acid and nucleotide availability for cell cycle progression. Sci Rep 9:14065. https://doi.org/10.1038/s41598-019-50547-9
5. Koppula P, Zhuang L, Gan B (2021) Cystine transporter SLC7A11/xCT in cancer: ferroptosis, nutrient dependency, and cancer therapy. Protein Cell 12:599–620. https://doi.org/10.1007/s13238-020-00789-5
6. Cantor JM, Ginsberg MH (2012) CD98 at the crossroads of adaptive immunity and cancer. J Cell Sci 125:1373–1382. https://doi.org/10.1242/jcs.096040
7. Kaira K, Oriuchi N, Imai H et al (2009) CD98 expression is associated with poor prognosis in resected non-small-cell lung cancer with lymph node metastases. Ann Surg Oncol 16:3473–3481. https://doi.org/10.1245/s10434-009-0685-0
8. Kaira K, Sunose Y, Arakawa K et al (2012) Prognostic significance of L-type amino-acid transporter 1 expression in surgically resected pancreatic cancer. Br J Cancer 107:632–638. https://doi.org/10.1038/bjc.2012.310
9. Canup BSB, Song H, Laroui H (2020) Role of CD98 in liver disease. Ann Hepatol 19:602–607. https://doi.org/10.1016/j.aohep.2019.11.011
10. Digomann D, Kurth I, Tyutyunnykova A et al (2019) The CD98 heavy chain is a marker and regulator of head and neck squamous cell carcinoma radiosensitivity. Clin Cancer Res 25:3152–3163. https://doi.org/10.1158/1078-0432.CCR-18-2951
11. Kawasaki Y, Omori Y, Suzuki S, Yamada T (2023) CD98hc as a marker of radiotherapy-resistant cancer stem cells in head and neck squamous cell carcinoma. Arch Med Sci 19:1859–1868. https://doi.org/10.5114/aoms.2020.92872
12. Hayes GM, Chinn L, Cantor JM et al (2015) Antitumor activity of an anti-CD98 antibody. Int J Cancer 137:710–720. https://doi.org/10.1002/ijc.29415
13. Choi HS, Lee HM, Kim MK, Ryu CJ (2022) Role of heat shock protein 60 in primed and naive states of human pluripotent stem cells. PLoS ONE 17:e0269547. https://doi.org/10.1371/journal.pone.0269547
14. Kim WT, Ryu CJ (2017) Cancer stem cell surface markers on normal stem cells. BMB Rep 50:285–298. https://doi.org/10.5483/bmbrep.2017.50.6.039
15. Seo SR, Lee HM, Choi HS, Kim WT, Cho EW, Ryu CJ (2017) Enhanced expression of cell-surface B-cell receptor-associated protein 31 contributes to poor survival of non-small cell lung carcinoma cells. PLoS ONE 12:e0188075. https://doi.org/10.1371/journal.pone.0188075
16. Lee HM, Seo SR, Kim J, Kim MK, Seo H, Kim KS, Jang YJ, Ryu CJ (2020) Expression dynamics of integrin alpha2, alpha3, and alphaV upon osteogenic differentiation of human mesenchymal stem cells. Stem Cell Res Ther 11:210. https://doi.org/10.1186/s13287-020-01714-7
17. Kim MK, Choi MJ, Lee HM, Choi HS, Park YK, Ryu CJ (2021) Heterogeneous nuclear ribonucleoprotein A2/B1 regulates the ERK and p53/HDM2 signaling pathways to promote the survival, proliferation and migration of non-small cell lung cancer cells. Oncol Rep. https://doi.org/10.3892/or.2021.8104
18. Wang Z, Raifu M, Howard M, Smith L, Hansen D, Goldsby R, Ratner D (2000) Universal PCR amplification of mouse immunoglobulin gene variable regions: the design of degenerate primers and an assessment of the effect of DNA polymerase 3' to 5' exonuclease activity. J Immunol Methods 233:167–177. https://doi.org/10.1016/s0022-1759(99)00184-2
19. Yoon SO, Lee TS, Kim SJ et al (2006) Construction, affinity maturation, and biological characterization of an anti-tumor-associated glycoprotein-72 humanized antibody. J Biol Chem 281:6985–6992. https://doi.org/10.1074/jbc.M511165200
20. Sun JH, Luo Q, Liu LL, Song GB (2016) Liver cancer stem cell markers: progression and therapeutic implications. World J Gastroenterol 22:3547–3557. https://doi.org/10.3748/wjg.v22.i13.3547
21. Suresh R, Ali S, Ahmad A, Philip PA, Sarkar FH (2016) The role of cancer stem cells in recurrent and drug-resistant lung cancer. Adv Exp Med Biol 890:57–74. https://doi.org/10.1007/978-3-319-24932-2_4
22. Weiswald LB, Bellet D, Dangles-Marie V (2015) Spherical cancer models in tumor biology. Neoplasia 17:1–15. https://doi.org/10.1016/j.neo.2014.12.004
23. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828
24. Xia P, Dubrovska A (2023) CD98 heavy chain as a prognostic biomarker and target for cancer treatment. Front Oncol 13:1251100. https://doi.org/10.3389/fonc.2023.1251100
25. Hasegawa K, Ikeda S, Yaga M et al (2022) Selective targeting of multiple myeloma cells with a monoclonal antibody recognizing the ubiquitous protein CD98 heavy chain. Sci Transl Med. 14:eaxx7706. https://doi.org/10.1126/scitranslmed.aax7706
26. Manzo G (2019) Similarities between embryo development and cancer process suggest new strategies for research and therapy of tumors: a new point of view. Front Cell Dev Biol 7:20. https://doi.org/10.3389/fcell.2019.00020
27. Barati M, Akhondi M, Mousavi NS, Haghparast N, Ghodsi A, Baharvand H, Ebrahimi M, Hassani SN (2021) Pluripotent stem cells: cancer study, therapy, and vaccination. Stem Cell Rev Rep 17:1975–1992. https://doi.org/10.1007/s12015-021-10199-7
28. Tan HL, Choo A (2019) Opportunities for antibody discovery using human pluripotent stem cells: conservation of oncofetal targets. Int J Mol Sci. https://doi.org/10.3390/ijms20225752
29. Tan HL, Yong C, Tan BZ et al (2018) Conservation of oncofetal antigens on human embryonic stem cells enables discovery of monoclonal antibodies against cancer. Sci Rep 8:11608. https://doi.org/10.1038/s41598-018-30070-z
30. Montero JC, Del Carmen S, Abad M, Sayagues JM, Barbachano A, Fernandez-Barral A, Munoz A, Pandiella A (2023) An amino acid transporter subunit as an antibody-drug conjugate target in colorectal cancer. J Exp Clin Cancer Res 42:200. https://doi.org/10.1186/s13046-023-02784-0
31. Montero JC, Calvo-Jimenez E, Del Carmen S, Abad M, Ocana A, Pandiella A (2022) Surfaceome analyses uncover CD98hc as an antibody drug-conjugate target in triple negative breast cancer. J Exp Clin Cancer Res 41:106. https://doi.org/10.1186/s13046-022-02330-4
32. Rossier G, Meier C, Bauch C, Summa V, Sordat B, Verrey F, Kuhn LC (1999) LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. J Biol Chem 274:34948–34954. https://doi.org/10.1074/jbc.274.49.34948

33. Nakamura E, Sato M, Yang H et al (1999) 4F2 (CD98) heavy chain is associated covalently with an amino acid transporter and controls intracellular trafficking and membrane topology of 4F2 heterodimer. J Biol Chem 274:3009–3016. https://doi.org/10.1074/jbc.274.5.3009
34. Posey AD Jr, Schwab RD, Boesteanu AC et al (2016) Engineered CAR T cells targeting the cancer-associated Tn-glycoform of the membrane mucin MUC1 control adenocarcinoma. Immunity 44:1444–1454. https://doi.org/10.1016/j.immuni.2016.05.014
35. Hosen N, Matsunaga Y, Hasegawa K et al (2017) The activated conformation of integrin beta(7) is a novel multiple myeloma-specific target for CAR T cell therapy. Nat Med 23:1436–1443. https://doi.org/10.1038/nm.4431