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Amino Acid Transport Associated to Cluster of Differentiation 98 Heavy Chain (CD98hc) Is at the Cross-road of Oxidative Stress and Amino Acid Availability*

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CD98hc functions as an amino acid (AA) transporter (together with another subunit) and integrin signaling enhancer. It is overexpressed in highly proliferative cells in both physiological and pathological conditions. CD98hc deletion induces strong impairment of cell proliferation *in vivo* and *in vitro*. Here, we investigate CD98hc-associated AA transport in cell survival and proliferation. By using chimeric versions of CD98hc, the two functions of the protein can be uncoupled. Although recovering the CD98hc AA transport capacity restores the *in vivo* and *in vitro* proliferation of CD98hc-null cells, reconstitution of the integrin signaling function of CD98hc is unable to restore *in vitro* proliferation of those cells. CD98hc-associated transporters (*i.e.* xCT, LAT1, and y^+LAT2 in wild-type cells) are crucial to control reactive oxygen species and intracellular AA levels, thus sustaining cell survival and proliferation. Moreover, in CD98hc-null cells the deficiency of CD98hc/xCT cannot be compensated, leading to cell death by ferroptosis. Supplementation of culture media with β-mercaptoethanol rescues CD98hc-deficient cell survival. Under such conditions null cells show oxidative stress and intracellular AA imbalance and, consequently, limited proliferation. CD98hc-null cells also present reduced intracellular levels of branched-chain and aromatic amino acids (BCAAs and ARO AAs, respectively) and induced expression of peptide transporter 1 (PEPT1). Interestingly, external supply of dipeptides containing BCAAs and ARO AAs rescues cell proliferation and compensates for impaired uptake of CD98hc/LAT1 and CD98hc/y^+LAT2. Our data establish CD98hc as a master protective gene at the cross-road of redox control and AA availability, making it a relevant therapeutic target in cancer.

Proliferative cells have an increased demand for nutrients such as glucose, AAs,[8] fatty acids, and vitamins. Heteromeric amino acid transporters are among several families of solute carriers (SLC Tables website) that mediate the influx or efflux of solutes (AAs among others) through the plasma membrane of mammalian cells. Heteromeric amino acid transporters are composed of a heavy (SLC3 family) and a light (L-type amino acid transporters (LATs) from SLC7 family) subunit, linked by a disulfide bridge (1). The heavy chain carries the complex to the plasma membrane (2), whereas the light chain constitutes

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[8] The abbreviations used are: AA, amino acid; CD98hc, cluster of differentiation 98 heavy chain; β-ME, β-mercaptoethanol; BCAA, branched-chain amino acid; ARO AA, aromatic amino acid; PEPT1, peptide transporter 1; SLC, solute carrier; LAT, L-type amino acid transporter; ES cell, embryonic stem cell; AA^+, cationic amino acid; AA^0, neutral amino acid; SAS, sulfasalazine; NAC, *N*-acetylcysteine; Vit E, vitamin E; H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; CHAC1, cation transport regulator homolog 1; RPLP0, ribosomal phosphoprotein, large, P0; Nrf2, nuclear factor erythroid 2-related factor; SNAT, sodium-coupled amino acid transporter; S6, ribosomal protein S6; eIF2α, eukaryotic initiation factor 2; ATF4, activating transcription factor 4; ER, endoplasmic reticulum; BCH, 2-aminobicyclo [2,2,1] heptane-2-carboxylic acid; CAT, cationic amino acid transporter; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; ED, ectodomain.

the catalytic part of the transporter (3, 4). CD98hc (aka SLC3A2, 4F2, FRP1), the only ubiquitously expressed heavy subunit of heteromeric amino acid transporters, can bind to any of six light subunits (LAT1, LAT2, xCT, y^+LAT1, y^+LAT2, and asc1), which confer substrate specificity to the heterodimer, referred to as CD98 (1). All together these transporters cover a broad substrate spectrum, including all essential amino acids. Most heteromeric amino acid transporters are obligatory antiporters, meaning they mediate the simultaneous translocation of two AAs across the membrane in opposite directions (in a 1:1 stoichiometry). As a consequence of this mechanism of transport CD98 heterodimers are unable to generate net AA import. However, their up-regulation can increase the uptake of specific AAs (in exchange with others), rendering the proper AA pool to support cell growth.

Besides its function as transporter, CD98hc behaves as a co-receptor of β integrins and amplifies their downstream signaling (5, 6). A large body of evidence implicates CD98hc in cell proliferation during physiological and pathological conditions (7–9). Furthermore, the lack of CD98hc in mouse embryonic stem (ES) cells (in which exon 1, encoding the transmembrane domain of CD98hc, is replaced by a neomycin cassette) blocks cell proliferation *in vivo* (6). The CD98hc function as integrin signaling enhancer is sufficient to partially rescue the *in vivo* proliferation defect of CD98hc-null ES cells (6).

In this study we analyzed the role of CD98hc-associated AA transport in cell proliferation and teratoma formation. Interestingly, CD98hc presents specific binding capacity domains; whereas the intracellular domain is necessary and sufficient for interactions with β1 integrins (thus regulating their signaling capacities), the ectodomain (ED) is required for AA catalytic subunit association (10). We show that impaired proliferation of previously generated CD98hc-null ES cells and ES-derived fibroblasts (6) is restored by expression of chimeras that bind the AA transport catalytic subunits. Furthermore, such chimeras are able to specifically promote all AA transport activities observed in wild-type (WT) cells (namely CD98hc/xCT, system x_c^-; CD98hc/LAT1, system L and CD98hc/y^+LAT2, system y^+L). Next, we established the biological consequences of deleting CD98hc-mediated AA transport activities and found that ES-derived fibroblasts cannot compensate this deletion. Thus, invalidation of xCT activity results in iron-dependent oxidative (non-apoptotic) cell death called ferroptosis (11, 12). Culture medium supplementation with β-ME prevents ferroptosis and restores cell survival. In such conditions CD98hc-deficient cells present: (i) accumulation of reactive oxygen species, ii) modulation of CD98hc-independent AA transporters and up-regulation of peptide transporter PEPT1, (iii) intracellular AA imbalance with dramatic increase in cationic AAs (AA^+) and neutral AAs (AA^0) but reduced levels of BCAAs and ARO AAs, and (iv) concomitant limited cell proliferation. An external supply of BCAAs and ARO AAs in the form of dipeptides rescues cell proliferation. Thus, only medium supplementations (with β-ME and BCAA- and ARO AA-containing dipeptides) can compensate for disrupted uptake of essential amino acids by CD98hc-dependent transport systems x_c^-, L, and y^+L. Taken together, our results highlight the critical role of CD98hc-associated AA transport for cell survival and proliferation. We show that CD98hc functions as an integrative and protective hub between oxidative stress and low AA availability.

Experimental Procedures

Cell Culture—Wild-type and CD98hc-null mouse ES cells as well as corresponding ES-derived fibroblasts were cultured in complete DMEM high glucose (Gibco) medium supplemented with 10% v/v FBS (HyClone), 20 mM Hepes, pH 7.3, 100 μM non-essential amino acids (Gibco), 2 mM L-glutamine (Gibco), and if not stated otherwise, 100 μM β-ME (Sigma) at 37 °C and 5% v/v CO_2 in an humidified incubator.

Induction of Teratomas—A suspension of ES cells (1.5×10^6 cells per site) was injected subcutaneously into athymic BALB/c WEHI nude mice. After 33 days, teratomas were recovered and measured. To ensure similar expression levels in reconstitution experiments, each cell line was supplemented with CD98hc-null ES cells so that a similar number of expressing cells was injected with each clone.

Cell Proliferation Assay—On day 0, wild-type and CD98hc-null ES-derived fibroblasts were seeded in duplicate at 1×10^4 cells per 35-mm diameter dish. After 24 h of growth in complete supplemented DMEM medium (detailed above), cells were washed twice with PBS and put to grow in DMEM media with the corresponding additional supplementations (1 mM sulfasalazine (SAS), 1 mM *N*-acetylcysteine (NAC), 1 μg/ml vitamin E (Vit E), or BCAA- and ARO AA-containing dipeptides (see below) as indicated).

Flow Cytometry with 2′,7′-Dichlorodihydrofluorescein Diacetate (H_2DCFDA)-stained Cells—Briefly, cells were plated and cultured in normal conditions. Forty-eight hours after plating cells were washed with Hanks' balanced salt solution (HBSS) once and incubated with 1 μM H_2DCFDA (Molecular Probes) for 30 min at 37 °C. Then cells were harvested and washed in Hanks' balanced salt solution, and levels of intracellular fluorescence (non-fluorescent H_2DCFDA is converted to highly fluorescent 2′,7′-dichlorofluorescein (DCF) upon cleavage of the acetate groups by intracellular esterases and oxidation) were analyzed using a FACS Calibur.

Quantitative PCR/RNA Preparation—RNAs were extracted from cultured ES-derived fibroblasts using TRIzol reagent (Gibco). Reverse transcription was performed using Superscript II reverse transcriptase (Invitrogen). Sets of specific primers (cation transport regulator homolog 1 (CHAC1), sense (5′-CTTGAAGACCGTGAGGGCTG-3′), antisense (5′-GTGGGGTGGCCACATAGG); PEPT1, sense (5′-GCCGGACCAGATGCAGACGG-3′), antisense (5′-GCGGGTACACCACAGCGTCC-3′)) were used for amplification using 7900 HT Real Time PCR System (Applied Biosystems). Samples were normalized to ribosomal phosphoprotein, large, P0 (RPLP0) (sense, 5′-CACTGGTCTAGGACCCGAGAAG-3′; antisense, 5′-GGTGCCTCTGGAGATTTTCG-3′), using the ΔCt method. Statistical significance was determined by means of Student's *t* test.

AA Uptake Measurement—Transport activities were tested on whole cells as previously described (3) by measuring the transport of corresponding radiolabeled AA (10 μM). Transport activities were confirmed using 1 mM specific inhibitors (and pretreating cells with 1 mM *N*-ethylmaleimide for 3 min when needed) as indicated in the figure legends. (See Figs. 3 and 4.)

Western Blot—Whole cell lysates were prepared in radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 10 mM Tris, pH 7.2, 0.1% w/v SDS, 1% w/v Triton X-100, 1% w/v deoxycholate, 5 mM EDTA, 1 mM $NaVO_4$, 5 mM NaF, 1 mM PMSF, and protease inhibitor mixture (Roche Applied Science)) and centrifuged at 10,000 × *g* for 15 min at 4 °C. Protein lysates were quantified using the bicinchoninic acid (BCA) method (Thermo Scientific Pierce). Proteins were loaded (10 μg of total protein per lane) and resolved in 10% w/v or 12.5% w/v acrylamide gels by SDS-PAGE and transferred to Immobilon membranes (Millipore). They were then immunoblotted (see below) and detected by the ECL method.

Primary Antibodies—The following primary antibodies were used and validated for protein immunoblotting. (i) Anti-Nrf2 is a rabbit polyclonal antibody against synthetic peptide within human nuclear factor erythroid 2-related factor (Nrf2) C terminus; Abcam #ab62352 lot #GR149891–2; 1:2500; used to recognize mouse Nrf2. This antibody revealed a single band (with same mobility as the one validated in a previous report (13)) that responded to Nrf2 up-regulation in CD98hc-null cells treated with 25 μM *tert*-butylhydroquinone for 2 h. (ii) Anti-SNAT1 is a rabbit polyclonal antibody raised against human sodium-coupled amino acid transporter 1 (SNAT1); Santa Cruz #sc-67080 lot #G0208; 1:500. It reacts against mouse SNAT1, with several bands appearing in the film. Specificity of the ~50-kDa band was validated by its distinct disappearance after SNAT1 silencing in ES-derived wild-type fibroblasts and in mouse embryonic fibroblasts after lentivirus-mediated transduction of SNAT1 shRNA (Sigma, #TRCN0000069230) compared with control transduction with scrambled RNA (Sigma, #SHC00). (iii) Anti-p-S6 is a rabbit polyclonal antibody against a synthetic phosphopeptide corresponding to residues surrounding Ser-235 and Ser-236 of human ribosomal protein S6 (S6); Cell Signaling #2211S lot #22 Ref.02/2014; 1:2000. It reacts with S6 only when phosphorylated at serine 235 and 236 as shown in the datasheet. This antibody detected a single band, which decreased after treatment of murine fibroblasts with 2 μM rapamycin for 3 h, proving its specificity. (iv) Anti-S6 is a mouse monoclonal antibody against human S6; Santa Cruz #sc-74459 lot #G0708; 1:8000. This antibody reacts specifically against murine S6 as shown by the presence of a single band in the gels, corresponding to the size of the band revealed by anti-p-S6 but not responding to treatment with rapamycin. (v) Anti-p-eIF2α is a rabbit polyclonal antibody against a synthetic phosphopeptide corresponding to residues surrounding Ser-51 of the α subunit of human eukaryotic initiation factor 2 (eIF2); Cell Signaling #9721 lot #9 Ref.04/2012; 1:1000. Specificity against murine eIF2α was validated by up-regulation of phosphorylated-eIF2α after murine fibroblasts treatment with 10 nM thapsigargin for 3 h. (vi) Anti-eIF2α is a rabbit polyclonal antibody produced by immunizing animals with a synthetic peptide derived from the C-terminal sequence of eIF2α; Cell Signaling #9722 lot #13 Ref.04/2013; 1:1000. It was used to recognize murine total eIF2α. Specificity was validated by the presence of a unique band in the gels with the same size as p-eIF2α but not responding to thapsigargin treatment. (vii) Anti-ATF4 is a rabbit monoclonal antibody against human activating transcription factor 4 (ATF4); Cell Signaling D4B8 #11815 lot # 2; 1:1000. It was validated by up-regulation of the protein after thapsigargin treatment. (viii) Anti-PEPT1 is a custom made rabbit polyclonal antibody against human PEPT1. It was kindly provided and validated by Dr. H. Daniel (14, 15); 1:5000. (ix) Anti-tubulin (Sigma #T5168 lot #103M4773V; 1:1000) was used as a loading control, and correlation between total protein loaded and tubulin signal was checked.

Intracellular AA Quantification—Pelleted cells were weighed, and norleucine was added as an internal standard. Samples were deproteinized with 10% v/v trifluoroacetic acid and lysed by repeated freeze-thaw cycles. After centrifugation at maximum speed, supernatants were ultrafiltered through a 10-kDa spin column (Millipore) and dried using a vacuum chamber. Pellets were resuspended in loading buffer (Biochrom Ltd.), filtered, and injected for HPLC analysis. Quantitative analysis of AAs was performed using a Biochrom 30 amino acid analyzer (Biochrom Ltd.). AA peaks were identified on the basis of the retention times of the corresponding standards. Quantification was normalized by total protein.

Dipeptide Synthesis and Supplementation—BCAA- or ARO AA- and L-Ala dipeptides were synthesized following standard procedures by the ICTS "NANBIOSIS," more specifically by the Synthesis of Peptides Unit (U3) of the CIBER in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN) at the Barcelona Science Park. Briefly, dipeptides were synthesized in solution by coupling of the corresponding protected N- and C-terminal AAs using 2-(1H-benzotriazol-1-yl)-1,1,3-tetramethylmethyluronium hexafluoro phosphate (HBTU) in dimethylformamide as the coupling reagent. Once the reaction was finalized, protected dipeptides were precipitated with cold water, centrifuged, and washed again with cold water. Protecting groups were eliminated by the addition of trifluoroacetic acid, and after 1 h final dipeptides were precipitated by the addition on cold diethyl ether. Dipeptides were redissolved in H_2O and lyophilized. Purity was estimated by HPLC and HPLC-MS. Each dipeptide was supplemented at a concentration of 0.25× of the corresponding BCAA (isoleucine, leucine, and valine) or ARO AA (phenylalanine, tyrosine, and tryptophan) concentration in complete DMEM medium.

Results

CD98hc-associated AA Transport Function Is Sufficient to Support Cell Proliferation in Vivo—We previously found that (i) CD98hc-deficient ES cells fail to proliferate and form teratomas *in vivo* (6) and (ii) that the intracellular domain of CD98hc, required for enhanced β1 integrin signaling, rescues teratoma growth, although teratomas are as not as large as when CD98hc-null ES cells are reconstituted with wild-type CD98hc (6). Here we asked whether the partial teratoma growth observed could be ascribed to the lack of CD98hc-mediated AA transport function. Rescue experiments using CD98hc-CD69 chimeras (C69T98E98 and C98T69E98) (depicted in Fig. 1*A*) only able to bind CD98hc AA transport subunits but not integrins (10) showed restoration of *in vivo* cell proliferation. Importantly, reconstitution of CD98hc-null ES cells with wild-type CD98hc induced the strongest growth (Fig. 1, *B* and *C*). As expected, when CD98hc-null cells were reconstituted with CD69, no teratomas were formed (Fig. 1, *B*, *lower right panel*,

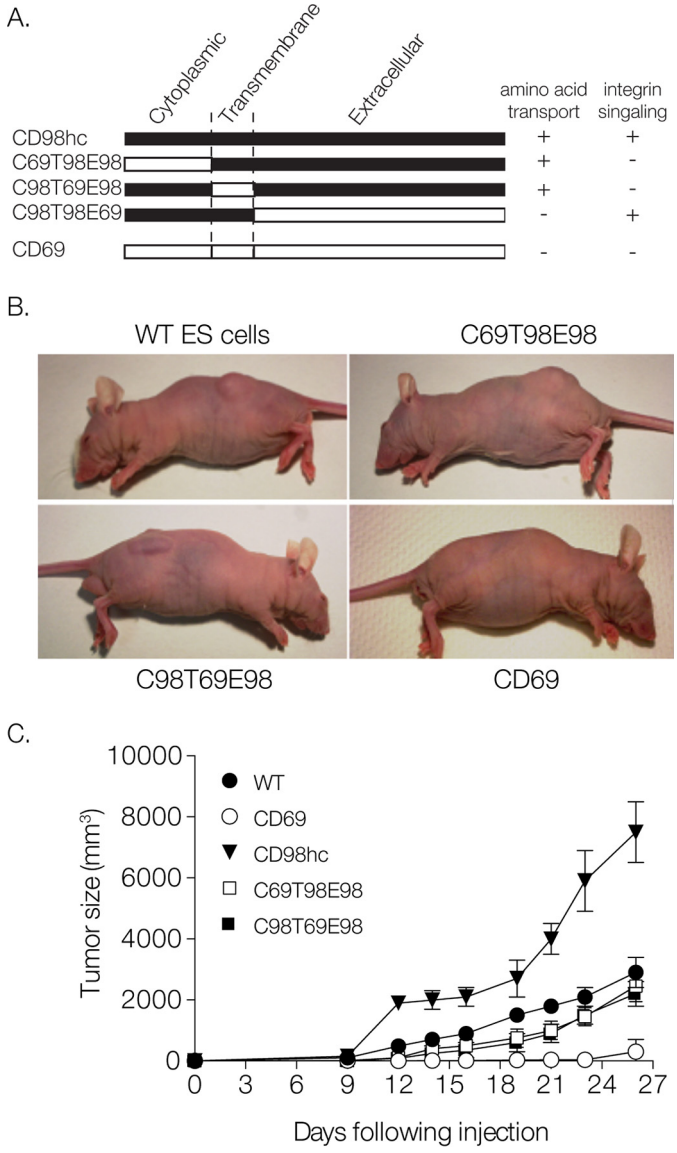

FIGURE 1. **CD98hc AA transport capacity restores cell proliferation *in vivo*.** *A*, depiction of chimeras of CD98hc and CD69 (type II transmembrane protein with functions unrelated to CD98hc) and their interactions with integrins or amino acid transporters. CD98hc protein is depicted in *black*, and CD69 is shown in *white*. Each chimera is defined by its cytoplasmic (*C*), transmembrane (*T*), and extracellular (*E*) domain derived from either CD98hc (*98*) or CD69 (*69*). *B*, mice were injected with corresponding ES cells and analyzed after 26 days. *C*, depicted is the quantification of tumor volumes (mean values ± S.E.) determined at various time points as previously described (6) for wild-type ES cells (●) and CD98hc-null ES cells reconstituted with CD69 (CD69, ○), full-length CD98hc (CD98hc, ▼), or with chimeras recovering only AA transport, namely C69T98E98 (□) or C98T69E98 (■). A similar expression level of full-length CD98hc and AA transport chimeras was confirmed by FACS (10).

and *C*). Thus, we show that (i) the portion of CD98hc that binds AA transporters promotes cell proliferation *in vivo* and (ii) maximal cell growth rates are only achieved when both CD98hc activities (AA transport and enhanced integrin signaling) are functional. These results suggest that both functions act in synergy to induce teratoma formation.

CD98hc Protects Cells against Oxidative Stress Leading to Cell Survival—Next, we analyzed the consequences of the lack of CD98hc-mediated AA transport activities. System x_c^- (CD98hc/xCT) is required for cystine uptake in exchange with glutamate. Hence, it plays a vital role in both redox control and cellular growth by supplying intracellular cysteine, a rate-limiting AA in the synthesis of GSH and necessary for protein synthesis (16). CD98hc-deficient ES-derived fibroblasts generated previously (6) did not survive under standard culture conditions (Fig. 2*A*, *left panel*) unless medium was supplemented with β-ME (Fig. 2*A*, *central panel*). xCT deficiency leads to ferroptosis. Up-regulation of endoplasmic reticulum (ER) stress response gene CHAC1 serves as a ferroptosis marker and can be reverted by adding β-ME to culture media (12), as shown in CD98hc-deficient cells (Fig. 2*B*) and in wild-type cells in which xCT transporter has been inhibited with sulfasalazine (*SAS*) (Fig. 2*B*). β-ME supplementation allowed reduction of extracellular cystine into free cysteine, which was subsequently imported by CD98hc-independent transporters (Fig. 2*A*, *right panel*), thus guaranteeing cell survival. We tested the effect of *N*-acetylcysteine (*NAC*) as an alternative source of cysteine and Vit E as an anti-oxidant (Fig. 2*C*) on cell survival. Vit E had no positive effect. In contrast, NAC protected CD98hc-null cells from death induced by the withdrawal of β-ME, thus rescuing cell proliferation (Fig. 2*C*). However, even when supplemented with β-ME, CD98hc-null fibroblasts showed a 1.4-fold increase of redox-sensitive probe H_2DCFDA labeling compared with wild-type fibroblasts (Fig. 2*D*). Because of the limitations in the reliability of such probe to accurately measure reactive oxygen species (17), we also analyzed in cells cultured in β-ME-supplemented medium the expression of the master regulator of intracellular antioxidant response Nrf2 (18). It presented indeed an increased expression (~9-fold) in CD98hc-null fibroblasts compared with the wild-type cells (Fig. 2*E*). In contrast, inhibition of xCT by SAS did not increase Nrf2 expression (Fig. 2*E*). Thus, CD98hc is required for *in vitro* cell survival because of its role in cystine uptake via x_c^- system, protecting cells from ferroptosis. Moreover, CD98hc ablation induces oxidative stress that is independent of xCT activity and non-reverted by β-ME.

CD98hc-mediated AA Transport (via xCT, LAT1, and y^+LAT2) Is Sufficient for Proper in Vitro Cell Proliferation—Both wild-type ES-induced teratomas (*in vivo*) and ES-derived fibroblasts (*in vitro*) express identical CD98hc-associated transporters as revealed by their mRNA expression (not shown): xCT (more expressed in cultured cells than *in vivo*, probably due to higher oxygen tension in culture than *in vivo* conditions (19)), LAT1, y^+LAT2, and to a lesser extent Asc1. Thus, the ES-derived fibroblasts provide a suitable *in vitro* model to establish the role of CD98hc-dependent AA transport in cell proliferation. Wild-type ES-derived fibroblasts presented transport activities corresponding to system x_c^-, system L, and system y^+L (Fig. 3*A*, *upper* and *middle panels*). CD98hc drives the trafficking of associated light chains to the plasma membrane (20). Thus, CD98hc-null cells should lack all CD98hc-associated transport subunits in the plasma membrane. Consistently, the system x_c^-, L, and y^+L transport activities were absent in CD98hc-deficient cells (Fig. 3*A*, *middle panel*). We show that the chimeras previously described to induce leucine transport (C69T98E98 and C98T69E98) (10) (Fig. 3*A*, *lower panel*) indeed recovered all CD98hc-mediated transport activities present in wild-type ES-derived fibroblasts (Fig. 3*A*, *middle panel*). In sharp contrast, the integrin-signaling

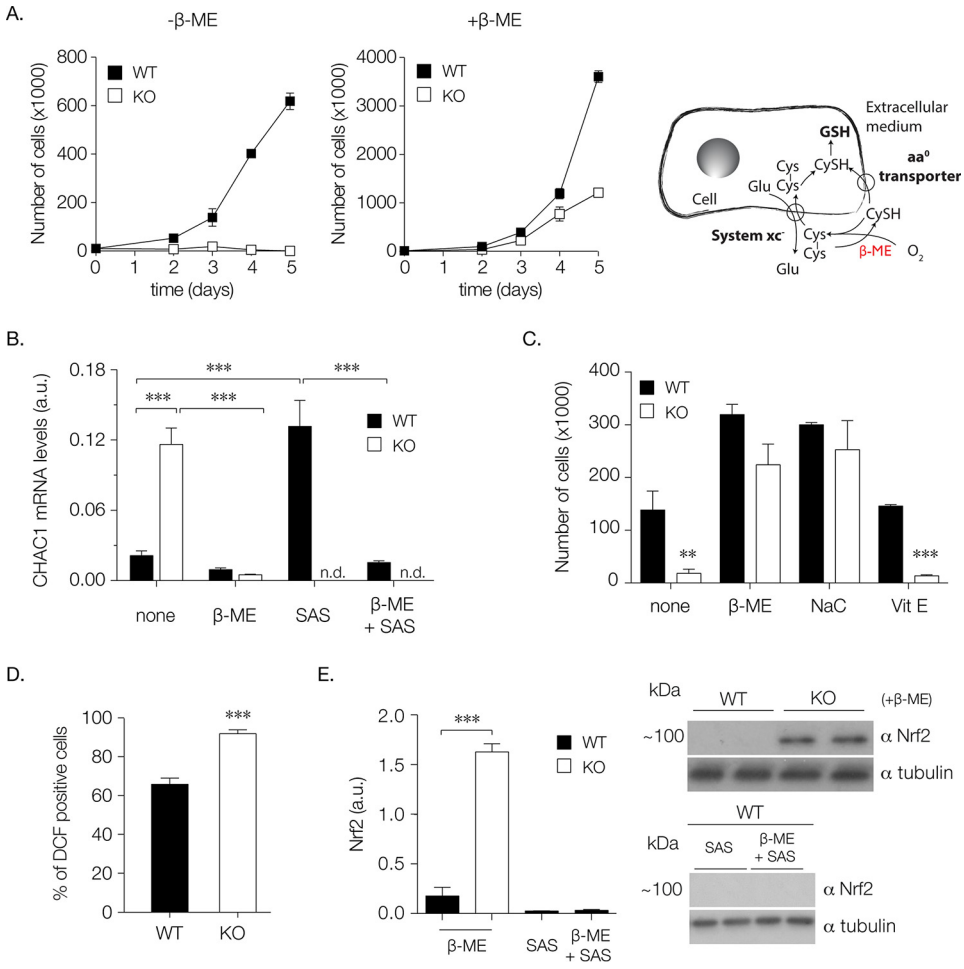

FIGURE 2. ***β*-Mercaptoethanol inhibits ferroptosis induced by depletion of CD98hc/xCT transporter and restores CD98hc-null cell survival.** *A*, WT and CD98hc-null (*KO*) fibroblast proliferation was compared in the absence (−) (*left panel*) and presence (+) (*middle panel*) of β-ME for 5 days. Cell counts at several time points are shown as the mean ± S.E. Similar results were obtained at least 3 times. Cystine (Cys-Cys) is transported by system x_c^- inside the cell where it is reduced to cysteine (CysSH, essential for the formation of reduced GSH). By supplementing the culture medium with β-ME, free CysSH is available extracellularly and can be imported inside the cell by CD98hc-independent neutral AA transporters (*right panel*). *B*, CHAC1 mRNA expression levels in WT (*solid bar*) and CD98hc-deficient fibroblasts (*KO*, *open bar*) grown with no additives (*none*) and in the presence of 100 μM β-ME, 1 mM SAS, or the combination of both (*β-ME + SAS*). RPLP0 was used as a housekeeping gene (not depicted). *n.d.*, not determined. Data are presented as the mean ± S.E. of eight independent experiments. *a.u.*, arbitrary units. *C*, WT (*solid bar*) and CD98hc-deficient (*KO*, *open bar*) fibroblasts were grown with no additives (*none*) or with 100 μM β-ME, 1 mM NAC, or 1 μg/ml Vit E for 48 h. Cell counts are expressed as the mean ± S.E. of duplicates. A representative experiment is shown; similar results were obtained at least 3 times. *D*, percentage of cells positive for the free radical sensor H_2DCFDA measured by flow cytometry. Data shows the mean ± S.E. of 12 measurements. E, Nrf2 protein expression in WT (*solid bar*) and CD98hc-null fibroblasts (*KO*, *open bar*) grown in the presence of 100 μM β-ME, 1 mM SAS, or the combination of both (*β-ME + SAS*). A representative Western blot of two independent experiments is shown. Data are normalized by tubulin expression. Quantification corresponds to the mean ± S.E. of six measurements. **, $p < 0.01$; ***, $p < 0.001$; Student's *t* test.

chimera (CD98T98E69) (Fig. 3*A*, *lower panel*) cannot mediate AA transport (Fig. 3*A*, *middle panel*). Next, to specifically study the effect of CD98hc-mediated AA transport on intrinsic cell proliferation capacity, we compared the *in vitro* cell proliferation rate of CD98hc-null fibroblasts when rescued with chimeras (described above) capable of restoring AA transport or integrin binding (10). First, we show that CD98hc-deficient cells present a major delay in proliferation *in vitro* compared with wild-type cells. This delay was rescued by reconstitution with full-length CD98hc (Fig. 3*B*, *upper panel*). Second, we observed a restoration of *in vitro* cell proliferation comparable with wild-type or full-length CD98hc-expressing cells only when null cells were reconstituted with AA transport chimeras (C69T98E98 and C98T69E98) (Fig. 3*B*, *lower panel*). Conversely, when only the capacity of integrin signaling (C98T98E69) was reconstituted in CD98hc-null cells, *in vitro* cell proliferation was still impaired (Fig. 3*B*, *lower panel*). Thus, CD98hc AA transport capacity is sufficient to drive proliferation *in vitro*, whereas the function as integrin signaling enhancer is dispensable. These results highlight the essential requirement of CD98hc-dependent AA transport for cell proliferation regardless of the surrounding environment.

CD98hc Supports the Balance of Intracellular AA Content—Deficiency of CD98hc and associated transporters had an impact on the intracellular AA content of CD98hc-null fibroblasts (Fig. 4*A*). The cell content of both AA^+ (Arg, Lys, and His) and AA^0 (Ala, Ser, Asn, Gln, and Met) was increased (2.9–4.3-fold and 1.2–2.7-fold, respectively) in CD98hc-deficient compared with wild-type fibroblasts. In contrast, BCAAs (Val, Leu, and Ile) and ARO AAs (Phe and Tyr) showed an intracellular content decrease of 0.5–0.6-fold in CD98hc-null when compared with wild-type cells (Fig. 4*A*). Analysis of the trans-

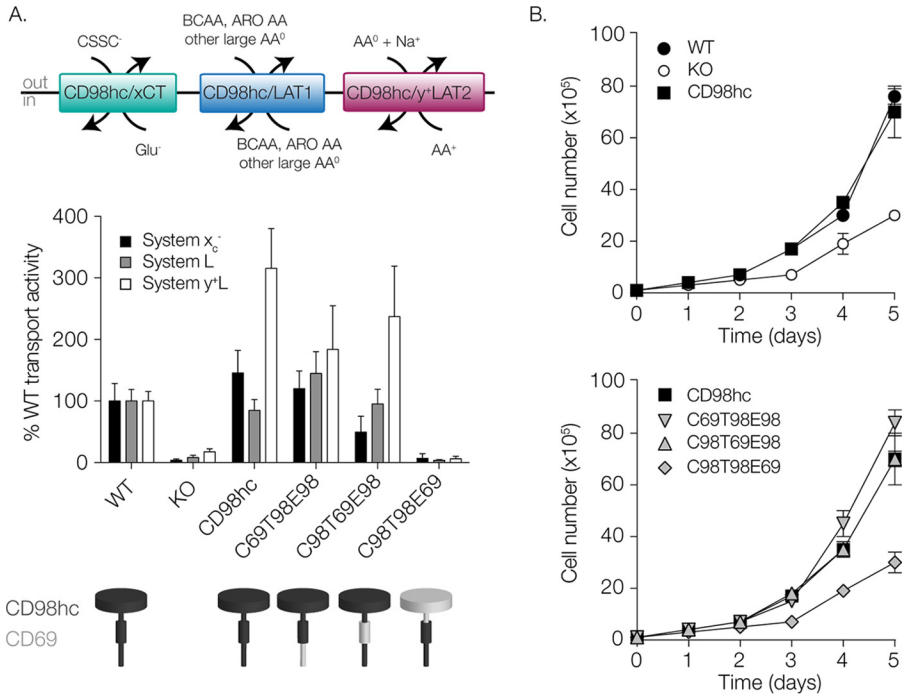

FIGURE 3. **CD98hc AA transport function is required for efficient cell proliferation *in vitro*.** *A*, depicted are the activities of transporters xCT (exchange of the anionic forms of cystine and glutamate), LAT1 (exchange of BCAAs, ARO AAs, and other large neutral AAs), and y^+LAT2 (exchange of cationic AAs and neutral AAs plus Na^+) across the plasma membrane (*upper panel*). AA transport activities of corresponding systems x_c^- (*black*), L (*gray*), and y^+L (*white*) in wild-type fibroblasts (*WT*), CD98hc-deficient (*KO*) fibroblasts, and CD98hc-null fibroblasts recovered with full-length CD98hc or chimeras recovering AA transport (C69T98E98 and C98T69E98) or integrin signaling (C98T98E69). Uptake activity is shown as a percentage of the levels of transport at 2 min (linear conditions) of wild-type cells for each CD98hc-associated transport system. Activities of the transport systems were determined as the uptake of the corresponding radiolabeled amino acid minus its uptake in the presence of specific inhibitor. Thus, system x_c^- represents the Na^+-independent uptake of L-Glu (10 μM) inhibitable by (*S*)-4-carboxyphenylglycine (1 mM); system L corresponds to the Na^+-independent L-Ile (10 μM) uptake that can be inhibited by 2-aminobicyclo [2,2,1] heptane-2-carboxylic acid (*BCH*) (1 mM), and system y^+L corresponds to the L-Arg (10 μM) uptake non-inhibitable by *N*-ethylmaleimide (1 mM) and inhibitable by L-Leu (1 mM) only when Na^+ is present. Transport data correspond to the mean ± S.E. of at least eight independent measurements (*middle panel*). Protein domains are depicted (CD98hc in *black*, CD69 in *gray*) (*lower panel*). *B*, Wild-type (WT, *black circle*) or CD98hc-deficient (*KO*, *white circle*) fibroblasts recovered with full-length CD98hc (*CD98hc*, *black square*) (*upper panel*) or CD98hc-null fibroblasts recovered with chimeras restoring AA transport (C69T98E98, *inverted grey triangle*, C98T69E98, *grey triangle*) or integrin signaling (C98T98E69, *grey diamond*) functions (*lower panel*, CD98hc is repeated as a reference) were seeded on day 0 and grown in the presence of β-ME for 5 days. Cells were counted every day. Results are expressed as the mean ± S.E. of duplicates. The experiment was repeated at least three times.

port of AAs with altered intracellular content revealed that, in the absence of CD98hc, the expression and activity of CD98hc-independent transporters was modulated. L-Arg uptake was much higher (~240%) in CD98hc-deficient than in wild-type ES-derived fibroblasts (Fig. 4*B*). We identified this transport activity as exclusively system y^+ (as *N*-ethylmaleimide specifically inactivates system y^+ without affecting system y^+L; Ref. 21), which presented an ~8-fold increase when compared with wild-type cells (Fig. 4*B*). Next, we tested whether the expression levels of cationic amino acid transporters (CATs) (system y^+) could account for such dramatically increased transport activity. In this regard we detected a 145-fold specific increase in CAT3 (but not CAT1 and CAT2) mRNA levels in CD98hc-null compared with wild-type fibroblasts (data not shown). Using the CAT3-specific inhibitor D-arginine (22), we measured an ~12-fold increase in CAT3 activity in CD98hc-null compared with wild-type cells (Fig. 4*C*). CAT3 is a CD98hc-independent transporter that mediates the Na^+-independent transport of cationic amino acids (1). Thus, the up-regulation of CAT3 in fibroblasts lacking CD98hc is in accordance with increased AA^+ intracellular content in such cells (Fig. 4*A*) as arginine, lysine, and histidine are substrates of murine CAT3 (22, 23). Regarding the transport of AA^0, by analyzing L-Ala uptake, we identified the presence of system A, a co-transporter of Na^+ and neutral AAs, mainly excluding BCAAs and ARO AAs (24) (Fig. 4*D*, *gray column*), system N, a Na^+-dependent transporter of neutral AAs (24) (Fig. 4*D*, *white column*), and others (part of L-Ala uptake was not ascribed to any specific transporter but could represent systems ASC, B^0 or $B^{0,+}$) (Fig. 4*D*, *black column*) in wild-type ES-derived fibroblasts. Depletion of CD98hc resulted in the disappearance of CD98hc-independent system A transport activity (Fig. 4*D*, *gray column*) along with the expression of SNAT1 transporter (Fig. 4*E*) (no differences in mRNA expression of SNAT2, the other system A isoform, were observed) (not shown). In contrast, system N was not changed in CD98hc-ablated cells (Fig. 4*C*, *white column*). System N couples AA import with H^+ efflux (1 AA: 1 Na^+ [symport]:1 H^+ [antiport]), making it less concentrative than system A (24). Despite the depletion of system A, the intracellular content of AA^0 (Ala, Ser, Asn, Gln, and Met) was increased in CD98hc-null cells (Fig. 4*A*). Thus, AA^0 accumulation would be generated by unidentified causes (*e.g.* reduced consumption rate) not related to AA^0 uptake. Ablation of CD98hc abrogated LAT1 transport activity (system L) (Fig. 3*A*, *middle panel*). LAT1 exchanges large AA^0, including BCAAs, ARO AAs, and histidine, methionine, and glutamine across the plasma membrane

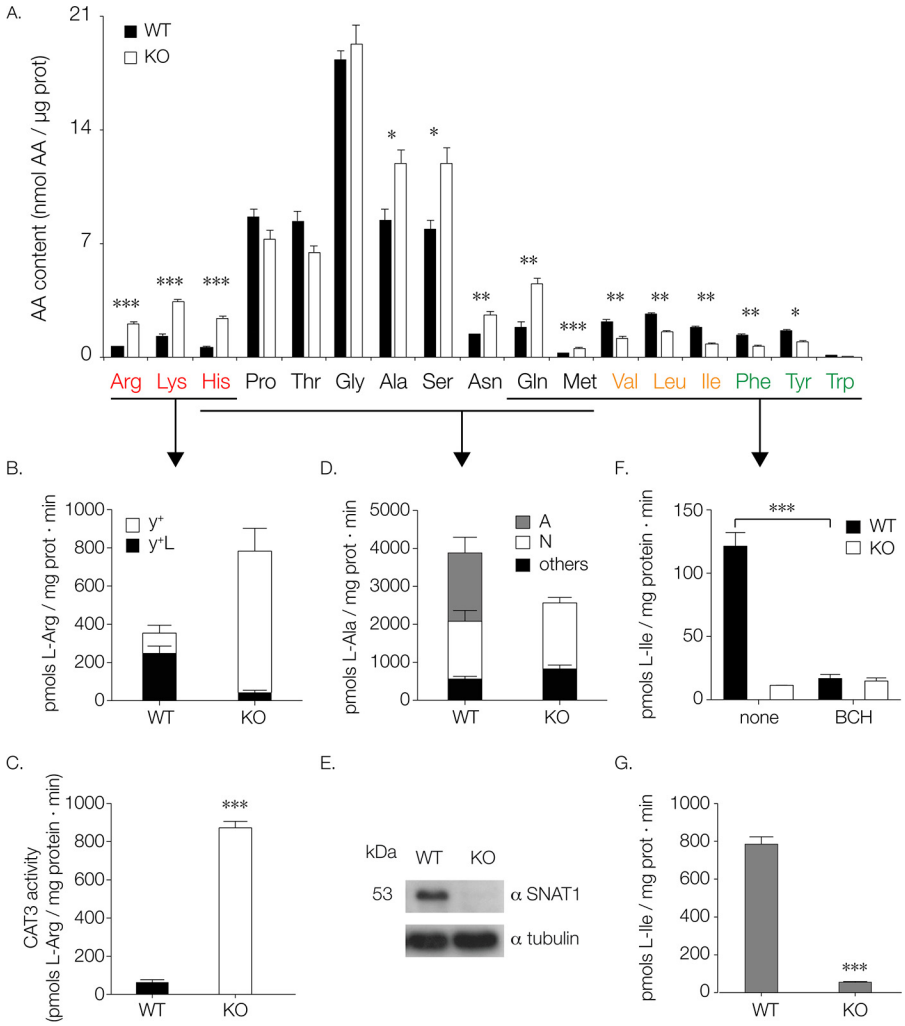

FIGURE 4. **CD98hc depletion leads to imbalanced intracellular AA content and to adaptations of CD98hc-independent transporters.** *A*, samples from WT (*solid bar*) and CD98hc-null (*KO*, *open bar*) fibroblasts were processed as described under "Experimental Procedures," and quantitative analysis of AAs was performed. Results are expressed as the mean ± S.E. of three independent measurements. AA^+ are presented in *red*, BCAAs are depicted in *yellow*, ARO AAs are in *green*, and the rest of AA^0 are in *black*. AAs are grouped and *underlined* to indicate that they are substrates of the corresponding transport system (below). *B*, Na^+-dependent L-Arg (10 μM) uptake at 2 min inhibitable by *N*-ethylmaleimide (1 mM) alone (system y^+, *white column*) or in combination with L-Leu (1 mM) (system y^+L, *black column*). Transport data correspond to the mean ± S.E. of at least 12 independent measurements. *C*, CAT3 transport activity measured as Na^+-independent uptake of L-Arg (10 μM) by WT (*solid bar*) and CD98hc-null (*KO*, *open bar*) fibroblasts inhibitable by D-Arg (5 mM) in the presence of L-Leu (1 mM). Transport data correspond to the mean ± S.E. of three independent experiments. *D*, Na^+-dependent L-Ala (100 μM) uptake at 2 min inhibitable by *N*-(methylamino)isobutyric acid (10 mM) alone (*system A*, *gray column*) or in combination with L-Asn (10 mM) (*system N*, *white column*). Residual transport is depicted as others (*black column*). Transport data correspond to the mean ± S.E. of at least eight independent measurements. *E*, protein expression of SNAT1 in WT and CD98hc-null (*KO*) fibroblasts. A representative Western blot is shown. Data are normalized by tubulin expression. Three independent experiments showed similar results. *F*, Na^+-independent L-Ile (10 μM) uptake at 2 min in the absence (none) and presence of BCH (1 mM) in WT (*solid bar*) and CD98hc-deficient (*KO*, *open bar*) fibroblasts. Transport data correspond to the mean ± S.E. of at least eight independent measurements. *G*, L-Ile (10 μM) uptake levels at 2 min in the presence of Na^+ in WT (*solid bar*) and CD98hc-null fibroblasts (*KO*, *open bar*). Transport data correspond to the mean ± S.E. of at least 12 independent measurements. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; Student's *t* test.

(25). System L has four isoforms, two associated with CD98hc (LAT1 and LAT2) and two independent of CD98hc (LAT3 and LAT4) (1, 26). All these transporters are Na^+-independent and can be inhibited by BCH, the leucine analog 2-aminobicyclo [2,2,1] heptane-2-carboxylic acid. Interestingly, in CD98hc-null cells, there was no Na^+-independent transport of L-Ile inhibitable by BCH (Fig. 4*F*). Thus, no CD98hc-independent isoform of system L compensated for LAT1 ablation. Moreover, CD98hc-deficient cells presented only ~10% of wild-type L-Ile uptake in Na^+-containing transport medium (Fig. 4*G*), thus arguing in favor of the absence of an efficient compensation for LAT1 by a Na^+-dependent transporter. Reduced intracellular BCAA and ARO AA content in CD98hc-null cells (Fig. 4*A*) is consistent with this lack of compensation for LAT1 transport and might underlie the proliferation defect of such cells.

Neither the Mechanistic Target of Rapamycin (mTOR) nor the Integrated Stress Response Is Responsible for CD98hc-null Cell Proliferation Deficiency—The mTOR and its downstream effector mTOR complex 1 (mTORC1) play a central role in cell growth and proliferation by responding to amino acid (*i.e.* leucine, arginine, glutamine) availability (27–30). mTORC1 phosphorylates and activates ribosomal protein S6 (S6) kinase that, in turn, mediates the phosphorylation of S6, an integral component of the 40S subunit of the ribosome (29, 31). In protein synthesis no AA can compensate for the absence of another;

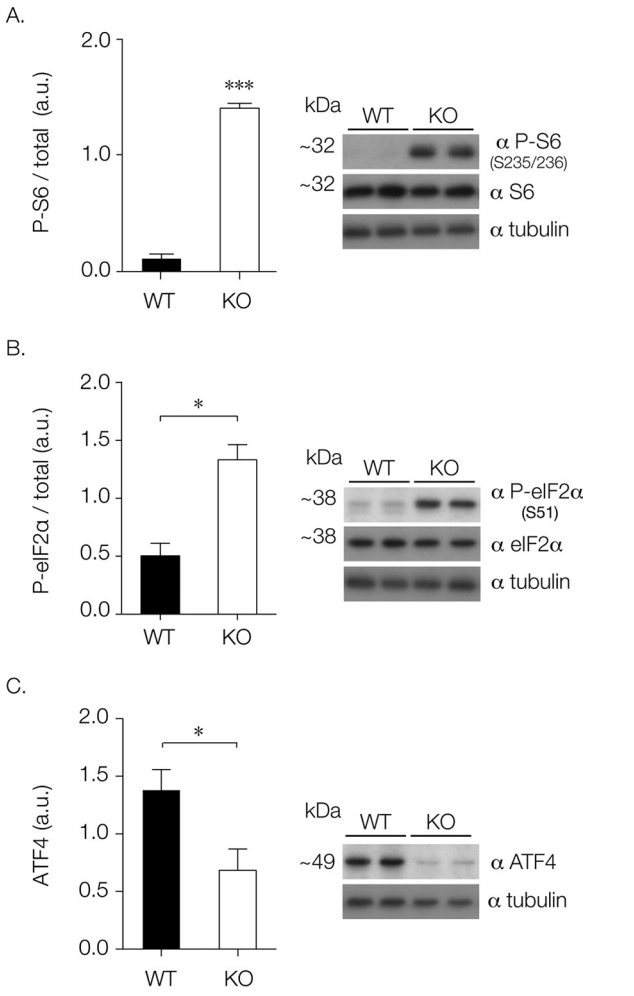

FIGURE 5. **Stress responses triggered in CD98hc-depleted fibroblasts.** *A*, mTORC1 is activated in CD98hc-deficient fibroblasts. Phosphorylation of S6 is compared between WT (*solid bar*) and CD98hc-null (*KO*, *open bar*) fibroblasts. *a.u.*, arbitrary units. *B*, eIF2α phosphorylation is compared between WT (*solid bar*) and CD98hc-null (*KO*, *open bar*) fibroblasts. *C*, ATF4 protein expression in WT (*solid bar*) and CD98hc-null (*KO*, *open bar*) cells. *A–C*, representative Western blots of two independent experiments are shown. Data are normalized by tubulin expression. Quantification corresponds to the mean ± S.E. of at least three independent experiments run in duplicates. *, $p < 0.05$; ***, $p < 0.001$; Student's *t* test.

thus, we thought that the intracellular BCAA and ARO AA shortage of CD98hc-deficient cells could induce protein synthesis repression via mTOR inactivation. Contrary to our hypothesis, we found an increased phosphorylation state of S6 in CD98hc-null compared with wild-type cells (Fig. 5*A*). In fact, increased activity of mTORC1 in fibroblasts deficient for CD98hc could be attributed to the high content of AA^+ and glutamine in such cells (Fig. 4*A*), as arginine and glutamine are also regulators of mTORC1 (32–34). Alternatively to mTORC1, eukaryotic cells respond to stress (such as oxidative stress or AA deprivation) by phosphorylating the α subunit of the eIF2, which represses global translation coincident with preferential translation of ATF4, a master regulator controlling the transcription of pro-survival target genes (35). This pathway is collectively referred to as integrated stress response. We analyzed the phosphorylation state of eIF2α in CD98hc-null cells, which was markedly increased (~3-fold) in comparison with wild-type cells (Fig. 5*B*), thereby suggesting the activation of the eIF2α-mediated stress response pathway. However, despite the strong phosphorylation of eIF2α, ATF4 mRNA (data not shown) and protein levels (Fig. 5*C*) were substantially repressed in CD98hc-deficient fibroblasts. Dysfunctional integrated stress response (suppressed ATF4 expression in the presence of phosphorylated eIF2α) has been reported in cells affected by chronic stresses (36–40). This could be the case for CD98hc-null ES-derived fibroblasts, which present chronic oxidative stress and imbalanced intracellular AA content. Moreover, CD98hc-deficient cell proliferation was restored without affecting the phosphorylation state of eIF2α (see below), offering serious doubts about the role of phosphorylated eIF2α in blocking general protein synthesis of ES-derived fibroblasts lacking CD98hc. In all, despite our attempts to decipher the molecular mechanisms behind, we cannot yet explain the cause for proliferation impairment in CD98hc-null cells.

CD98hc-deficient Cell Proliferation Is Restored after BCAA and ARO AA Supplementation—As shown above, in the absence of LAT1 activity and presenting only an ~10% of L-Ile uptake (Fig. 3*A*, *middle panel*, and Fig. 4*G*), CD98hc-deficient cells maintained a considerable (yet reduced) intracellular content of the LAT1 substrates valine, leucine, isoleucine, phenylalanine, and tyrosine (Fig. 4*A*). This observation suggests a diminished cell metabolism or alternative sources of these AAs. In this regard, we observed an overexpression of mRNA (Fig. 6*A*) and protein (Fig. 6*B*) levels of peptide transporter PEPT1 (whereas PEPT2 mRNA expression was not affected) (data not shown) in CD98hc-deficient fibroblasts when compared with wild-type fibroblasts. PEPT1 is a proton-dependent transporter responsible for cellular uptake of di- and tripeptides (41). We took advantage of this overexpression to supplement culture media of CD98hc-KO cells with alanyl dipeptides containing BCAAs and ARO AAs. Intriguingly, the proliferation capacity of CD98hc-deficient fibroblasts was restored to levels of wild-type cells after being grown in dipeptide-supplemented media for 5 days (Fig. 6*C*), whereas control-supplementation (L-Ala) showed no effect (Fig. 6*C*, *right panel*). Next, we performed a time-course analysis and found that up-regulation of PEPT1 expression matched the rescue of cell proliferation, with the highest mRNA and protein expression being reached at days 5–6 after cell seeding (data not shown). Therefore, cell proliferation was restored when fibroblasts expressed the transporter that allows extra uptake of dipeptides containing BCAAs and ARO AAs. Strikingly, the intracellular AA content of dipeptide-supplemented fibroblasts showed no recovery of either BCAA or ARO AA content (Fig. 6*D*); neither phosphorylation of eIF2α nor ATF4 repression was alleviated in the presence of BCAA- and ARO AA-containing dipeptides (data not shown). CD98hc-null ES-derived fibroblasts grown in peptide-supplemented media proliferated normally in the presence of phosphorylated eIF2α, supporting a dysfunctional role of the phosphorylation of the initiation factor on protein synthesis in these cells. Our results reveal the strong dependence of ES-derived fibroblasts on adequate BCAA and ARO AA availability for cell proliferation and the strict requirement of CD98hc/LAT1 and CD98hc/y^+LAT2 for mediating the transport of such essential AAs.

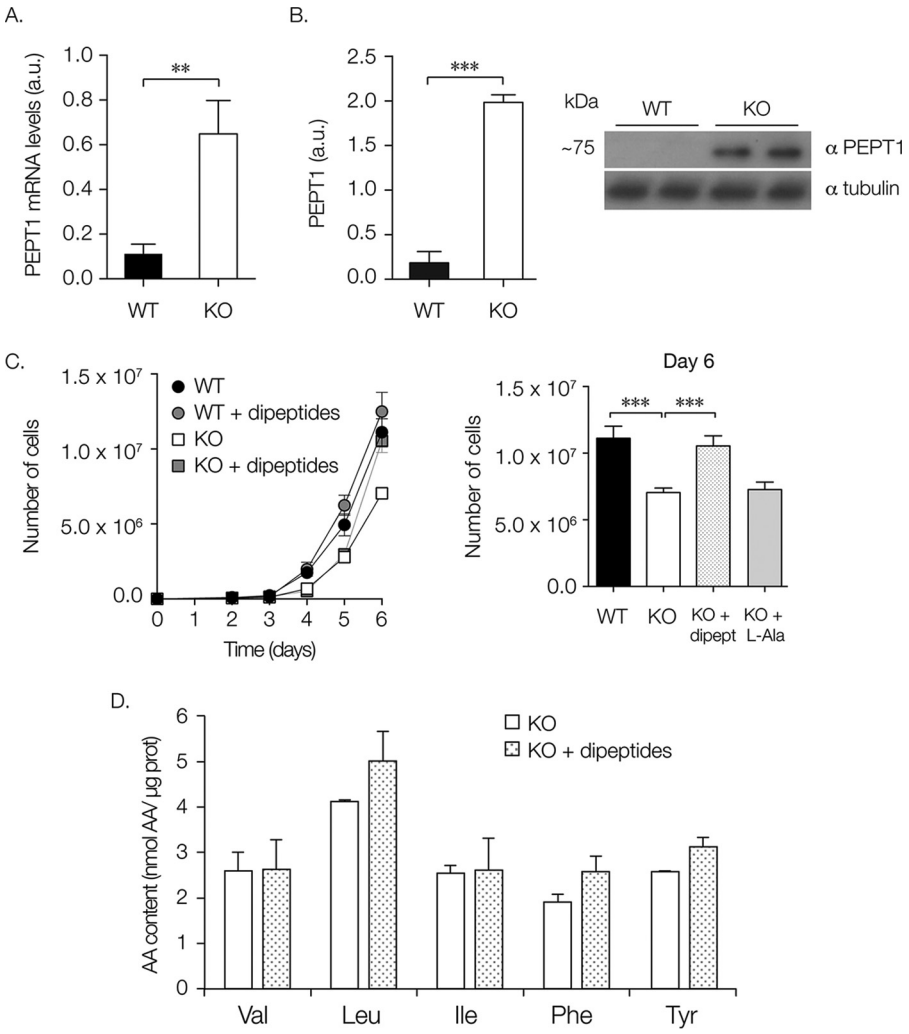

FIGURE 6. **Supplementation with BCAA- and ARO AA-containing dipeptides restores proliferation of CD98hc-null fibroblasts.** *A*, PEPT1 mRNA expression levels in WT and CD98hc-deficient (*KO*) fibroblasts. RPLP0 was used as a housekeeping gene (not depicted). Data are presented as the mean ± S.E. of three independent experiments. *a.u.*, arbitrary units. *B*, PEPT1 protein expression in WT and CD98hc-null (*KO*) fibroblasts. Representative Western blot of two independent experiments is shown. Data are normalized by tubulin expression. Quantification corresponds to the mean ± S.E. of eight measurements (*right panel*). *C*, proliferation of WT and CD98hc-null (*KO*) fibroblasts grown with no additives or in the presence of BCAA- and ARO AA- containing dipeptides (*WT + dipeptides* and *KO + dipeptides*) was measured over 6 days. Cell counts at several time points are shown as the mean ± S.E. of three independent experiments (*left panel*). Cell count at day 6 including KO + Ala as control (*right panel*). *D*, samples from CD98hc-null (*KO*, *open bar*) and CD98hc-null + dipeptides (*KO + dipeptides*, *dotted open bar*) fibroblasts were processed as described under "Experimental Procedures," and quantitative analysis of BCAAs and ARO AAs was performed. Results are expressed as the mean ± S.E. of three independent measurements. **, $p < 0.01$; ***, $p < 0.001$; Student's *t* test.

Discussion

Proliferating cells must meet specific energetic and biosynthetic demands (42). Their adaptation involves the expression of nutrient transporters, in particular for AAs that cells are unable to store. In this study we unveil the role of CD98hc-mediated AA transport in teratoma formation and proliferation *in vivo*. We show that CD98hc AA transport function is required for adequate provision of AAs to cells, protecting them against oxidative stress and allowing normal cell proliferation, both *in vitro* and *in vivo*.

We show that expression of the CD98hc portion able to bind integrins (C98T98E69) in CD98hc-null cells is not sufficient to restore *in vitro* cell proliferation, in contrast with *in vivo* teratoma formation (6), suggesting a specific integrin mediated regulation depending on the properties of the surrounding environment (43). In parallel, expression of any of the chimeras deficient for integrin interaction but containing the CD98hc-ED (C69T98E98, C98T69E98) (6, 10) rescues *in vivo* and *in vitro* cell proliferation of CD98hc-deficient cells.

Our data suggest that the ED of CD98hc (absent in the chimera exclusively recovering β1 integrin signaling) is crucial for the proper functioning of associated transporters, as CD98hc-null cells expressing ED-containing chimeras catalyze all AA transport activities present in wild-type cells (systems x_c^-, L, and y^+L). This observation is in agreement with a recent structural model of CD98hc heterodimers, which shows that CD98hc-ED interacts with the corresponding associated transporter, thus stabilizing it (4).

Distinct AA transporters often share the same substrates, thereby ensuring that the system remains unaffected if one transporter fails. Here we report a general change in intracellular AA content after invalidation of a transporter component, showing that redundancy is not observed with CD98hc-associated transporters. Consequently, CD98hc/

xCT and CD98hc/LAT1 (up-regulated in highly proliferative cells; Ref. 44) and CD98hc/y^+LAT2 are essential for cell survival and proliferation.

In vitro in high oxygen tension, cells rely on membrane expression of CD98hc-associated cystine transporter xCT (20), which protects them from cell death by ferroptosis (11). We show that CD98hc-null ES-derived fibroblasts do not survive under routine culture conditions but grow normally in the presence of β-ME or NAC. Our results are in good agreement with the work by Sato *et al.* (45) on fibroblasts isolated from xCT deficient embryos. However, whereas Vit E restores cell survival in xCT-null fibroblasts (45), it has no effect on CD98hc-deficient fibroblasts. This aggravated phenotype might be due to the lack of other transporters (LAT1 and y^+LAT2) along with xCT in CD98hc-null cells. Moreover, even when ferroptosis is blocked by β-ME supplementation (consistently with Ref. 12), CD98hc-null fibroblasts still present, with no obvious explanation, accumulation of 2′,7′-dichlorofluorescein signal and increased Nrf2 expression. Although it is known as a master regulator of oxidative stress, Nrf2 also participates in intermediary metabolism and mitochondrial physiology (46). Because pharmacological inhibition of xCT does not promote Nrf2 activation, one might question whether the observed increased Nrf2 in CD98hc-nulls is directly related to oxidative stress. Further investigation will be needed to clarify this process.

LAT1 is the other CD98 catalytic subunit overexpressed in highly proliferative cells (44). Although inhibition of LAT1 affects cell proliferation (47, 48), its genetic or chemical ablation results in mTOR inhibition and impaired cell growth (27, 28, 48–50). Our data indicate that >90% of L-isoleucine uptake is lost when CD98hc is absent. Moreover, CD98hc/LAT1 loss leads to cellular depletion of several essential AAs (BCAAs leucine, isoleucine, and valine and ARO AAs phenylalanine and tyrosine), which surprisingly does not impair mTORC1 activity. We hypothesize that the high intracellular content of other AAs such as arginine and glutamine is sufficient to sustain mTORC1 activity (as observed in Refs. 32–34) in CD98hc-null cells. Nonetheless, even with active mTORC1 CD98hc-null cells present a major defect in cell proliferation. In the absence of CD98hc, oxidative stress and/or scarcity of BCAAs and ARO AAs may underlie eIF2α phosphorylation; however, p-eIF2α does not appear to play a relevant role in the restriction of general translation of CD98hc-null ES-derived fibroblasts.

Isolated cells (both normal and malignant) depend on 13 AAs for *in vitro* survival. Over and above the eight AAs required for nitrogen balance (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), cell cultures require arginine, cysteine/cystine, glutamine, histidine, and tyrosine (51). Despite the absence of LAT1 activity, CD98hc-deficient cells maintain a considerable intracellular concentration of the BCAA and ARO AA substrates of LAT1. We could explain these somewhat unexpected results by the strong up-regulation of di- and tri-peptide transporter PEPT1 (41) expression in CD98hc-deficient cells. Providing dipeptides as an alternative source of BCAAs and ARO AAs is sufficient to recover proper cell proliferation. However, it neither alleviates stress sensed by eIF2α nor restores the intracellular BCAA and ARO

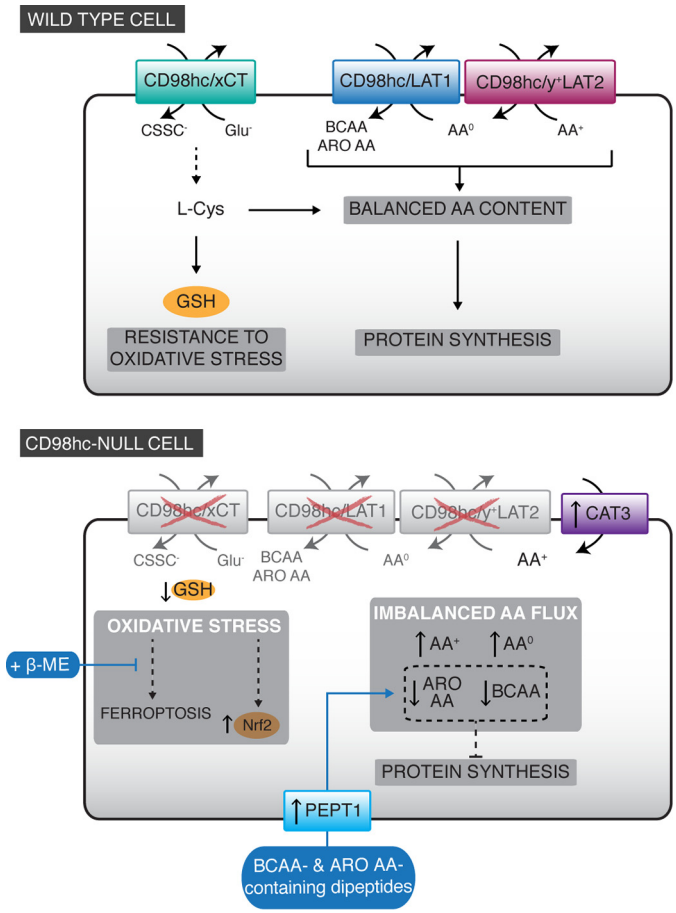

FIGURE 7. **CD98hc serves as a hub of the stress response network.** In wild-type fibroblasts (*upper panel*) CD98hc-associated transporters (LAT1, xCT, and y^+LAT2) ensure that cells have a balanced AA content, which allows them to counterbalance oxidative stress (via CD98hc/xCT) and to fuel protein synthesis and concomitant cell proliferation. When CD98hc is not present (*lower panel*), all associated transporters fail to reach the plasma membrane, and therefore, there is no effective AA transport activity of LAT1, xCT, and y^+LAT2 transporters. β-ME supplementation is then required to inhibit cell death by ferroptosis. In supplemented culture media, CD98hc-null cells present reactive oxygen species accumulation (triggering Nrf2) and imbalanced AA flux (increased concentration of AA^+ caused by up-regulation of CAT3 CD98hc-independent AA transporter, accumulation of AA^0, and shortage of BCAAs and ARO AAs), which lead to a defect in cell proliferation. External supply of BCAAs and ARO AAs in the form of dipeptides (which can enter the cell via PEPT1 di- and tripeptide transporter and compensate for the lack of CD98hc/LAT1 and CD98hc/y^+LAT2 transport activities) restores cell proliferation.

AA content, pointing to the relevance of AA flux rather than AA concentration. PEPT1 has been described to participate in tumor cell growth (52); our data now point to its relevant role in cell proliferation.

By controlling expression at the plasma membrane and coupling transport activities of xCT, LAT1, and y^+LAT2, CD98hc confers protection against oxidative and nutritional stresses (ensuring a balanced AA content and a proper supply of essential amino acids) and, thus, provides cells with a proliferative advantage (Fig. 7). Each function of CD98hc, AA transport activity (presented herein), or integrin signaling (6), rescues *in vivo* cell proliferation. Moreover, our results strongly suggest that both functions can act in synergy, providing cells with hyperproliferative capacity *in vivo* (as can be observed in teratomas formed when full-length CD98hc is expressed in CD98hc-null ES cells). These findings have relevant implica-

tions in pathophysiological scenarios. For instance, CD98hc and the associated catalytic subunits LAT1 and xCT, as well as integrins, are overexpressed in most tumors (8, 44, 53), thus promoting cell growth. By expressing such transporters coupled with extracellular matrix receptors, cells can control AA adequacy, reactive oxygen species impact, and anchoring/migration, leading to sustain cell proliferation, critical for tumor cells (54). To date, many studies have focused on the search for either transporter (xCT, LAT1) inhibitors or integrin antagonists as anti-cancer strategies (47, 48, 55, 56). Even though these molecules are appealing targets, encouraging, yet limited results, have been obtained so far. Targeting CD98hc could represent a novel option, widening the therapeutic window of cancer therapy by inducing an immediate blockage of AA transport activities mediated by xCT and LAT1 (with extremely limited compensation capacities) as well as a strong impairment in integrin signaling. CD98hc-targeting drugs could be delivered locally to tumors where this protein is strongly overexpressed. Generating drugs against CD98hc that could inhibit such a pivotal signal integrator would be of potential great use specifically for anti-cancer therapeutic purposes.

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