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ORIGINAL ARTICLE

Cathepsin G-mediated proteolytic degradation of MHC class I molecules to facilitate immune detection of human glioblastoma cells

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Abstract To mount an adaptive immune response, MHC I molecules present antigenic peptides to CTLs. Transcriptional reduction of MHC I molecules is a strategy of immune evasion, which impairs the detection of infected or tumorous cells by CTLs. Natural killer (NK) cells, on the other hand, eliminate target cells specifically in the absence of MHC I. Consequently, infected or tumorous cells partly retain their MHC I at the cell surface to avoid NK recognition. However, it remains unclear which protease degrades MHC I molecules and how these cells maintain a limited set of MHC I at the cell surface. Here, we demonstrate that cathepsin G (CatG), a serine protease, found in the endocytic compartment of APCs and, to a lesser extent, CatD and CatS proteolytically degrade MHC I molecules. Inhibition of CatG boosted MHC I expression at the cell surface of primary human immune cells. In contrast, human glioblastoma cells do not harbor active CatG and might have lost the ability to proteolytically degrade MHC I during tumorigenesis to avoid NK-mediated killing. Overexpression of CatG in glioblastoma cells resulted in a rapid and efficient MHC I degradation. In conclusion, CatG is an essential protease for regulating MHC I molecules and thus modulation of CatG activity might present a new avenue for therapeutic intervention.

Keywords MHC I · Cathepsin · Glioblastoma · Immune evasion

Abbreviations

BLC	B-lymphoblastoid cells
Cat	Cathepsin
CatGinh	Cathepsin G inhibitor
DCG-04	Doron C. Greenbaum 04
DTT	Dithiothreitol
E64	*trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane, L-*trans*-3-carboxy-oxiran-2-carbonyl-L-leucylagmatine, N-(trans-epoxysuccinyl)-L-leucine 4-guanidinobutylamide
E64d	(2S,3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester
ECL	Enhanced chemiluminescence
EGF	Epidermal growth factor
eGFP	Enhanced green fluorescent protein
ER	Endoplasmic reticulum
FGF	Fibroblast growth factor
HLA	Human leukocyte antigen
IGEPAL CA-630	Octylphenoxy-poly(ethyleneoxy) ethanol

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LHVS	Morpholinurea-leucine-homophenylalanine-vinylsulfone-phenyl
MARS116	Marcin Sienczyk 116
NCBI	National Center for Biotechnology Information
NK cells	Natural killer cells
PC	FBS-differentiated SC
PepA	Pepstatin A
PVDF	Polyvinylidene fluoride
SC	Sphere-cultured stem cell-enriched glioblastoma cell population

Introduction

Cell surface MHC molecules display the intracellular status of the respective cell. Before MHC molecules traffic to the cell surface, they are loaded with processed antigens from different sources. Cytosolic antigens are mainly shredded by the proteasome and the resulting antigenic peptides are directed to the endoplasmic reticulum (ER) to be further trimmed by exopeptidases. After peptide delivery onto the MHC I molecule, the MHC I-peptide complex is presented on the cell surface to CTLs [1]. Additionally, within the classical MHC I antigen processing and presentation pathway, cross talk between exogenous antigens and MHC I loading exists in dendritic cells, B cells, and macrophages. Cell surface MHC I molecules can be endocytosed (recycling pathway), ER-resident MHC I molecules can traffic via the trans-Golgi network to the endocytic compartment (vacuolar pathway), or reach the phagosome, where MHC I are loaded with a new set of exogenous/endocytic-derived antigenic peptides and reach the cell surface for inspection by patrolling CTLs or are degraded by a yet to be identified protease [2–5].

Tumor cells downregulate MHC I molecules to avoid elimination by CTLs which is pivotal for immune evasion [6]. Glioblastoma, for instance, is one of the most aggressive tumors, and one reason for treatment failure is the ability of glioblastoma cells to infiltrate the surrounding healthy tissue [7, 8]. Indeed, it has been demonstrated that invading/migrating glioblastoma cells partly downregulate MHC I as a strategy to evade CTL activation [9] by reducing levels of newly synthesized MHC I. On the other hand, glioblastoma cells still maintain a limited set of MHC I presumably to avoid recognition by natural killer (NK) cells [10].

It is not known which protease is responsible for the proteolytic regulation of MHC I molecules in the endocytic compartment (endosome/lysosome). We found that cathepsin G (CatG), a serine protease exhibited by distinct cell types including monocytes, degraded MHC I molecules. Consequently, inhibition of CatG boosted cell surface MHC I in PBMCs. Contrarily, HEK293T (proof of principle), glioblastoma cell lines, and a sphere-cultured stem cell-enriched glioblastoma cell population (SC), which do not express CatG, degraded and downregulated MHC I upon reconstitution of CatG expression. This highlights the pivotal role of CatG in orchestrating the MHC I antigen presentation machinery.

Materials and methods

In vitro processing of soluble MHC I

The heavy chain of HLA-A*02:01 and HLA-B*15:01 (Stefan Stevanovic, University of Tübingen, Germany) without transmembrane region and with a biotinylation site was expressed in E. coli and purified as previously described [11]. 0.1 µg/µl recombinant HLA-A*02:01, HLA-B*15:01, or HLA-C (Stratech Scientific Ltd, UK) was incubated with 4 ng/µl CatG from human sputum (Sigma-Aldrich, St. Louis, MO, USA) in the presence of 10 µM pepstatin A and 10 µM *trans*-epoxysuccinyl-L-leucylamido(4-guanidino) butane, L-*trans*-3-carboxyoxiran-2-carbonyl-L-leucylagmatine, N-(trans-epoxysuccinyl)-L-leucine 4-guanidinobutylamide (E64, Enzo Life Sciences GmbH, Lörrach, Germany), CatD (4 ng/µl, Enzo Life Sciences GmbH), or recombinant CatS (0.1 ng/µl, Enzo Life Sciences) for 2 h at 37 °C in reaction buffer (0.1 M citrate, pH 4.5–6.5). Some samples were preincubated with the CatG inhibitor I (10 µM, Calbiochem, Merck Chemicals GmbH, Schwalbach, Germany), pepstatin A (PepA, 10 µM, Enzo Life Sciences), or morpholinurea-leucine-homophenylalanine-vinylsulfone phenyl (LHVS, 10 nM, Michael Reich, Ulm University Medical Centre, Ulm, Germany) for 10 min at room temperature. The digestion patterns were resolved by SDS-PAGE and visualized by Coomassie blue staining.

In vitro processing and Western blot

B-lymphoblastoid cells (BLC) were cultured in medium (RPMI 1640, Life Technologies, Darmstadt, Germany) supplemented with 10 % FBS and antibiotics. Cells were lysed with 0.5 % octylphenoxy-poly(ethyleneoxy)ethanol (IGEPAL CA-630), 10 mM Tris pH 7.5, and 150 mM NaCl and 20 µg of BLC-derived cell lysate were incubated with 4 ng/µl human sputum CatG (Sigma-Aldrich) in 0.1 M citrate buffer (pH 4.5–6.5) for 2 h at 37 °C. Samples were boiled after addition of sample buffer including dithiothreitol (DTT), resolved with a 12 % SDS-PAGE, and proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (GE Healthcare, Freiburg, Germany). Afterwards, membranes were blocked for 1 h in blocking buffer (1× PBS, 0.05 % Tween 20, milk powder) and incubated

for 1 h with HLA-DR-specific antibody (CHAMP, Larry Stern, University of Massachusetts, MA, USA), anti-HLA-A (MyBioSource, San Diego, CA, USA), which detects HLA-A, HLA-B, and HLA-C, or anti-HLA-B (HC-10, Stefan Stevanovic, University of Tübingen, Germany). After washing (PBS with 0.05 % Tween 20), HRP-conjugated secondary antibody (GE Healthcare) was added for 1 h. Following additional washing steps, HRP activity was determined by enhanced chemiluminescence (ECL) detection kit (GE Healthcare). Bands were visualized using Hyperfilm ECL (GE Healthcare).

Assessment of MHC I surface expression

Freshly Ficoll-isolated human PBMCs (HLA-A*02:01/x) from heparinized blood or from cryo-preserved liquid nitrogen were incubated with CatG inhibitor I (10 µM, Calbiochem, Merck, Darmstadt, Germany) [12], (2S,3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester (E64d, 10 µM, Enzo Life Sciences), or DMSO in RPMI 1640 medium supplemented with 10 % FBS and antibiotics for 24 h at 37 °C. Subsequently, cells were washed in PBS containing 1 % FBS and stained with anti-HLA-A/B/C-APC (clone W6/32; eBioscience, San Diego, CA, USA) diluted in blocking buffer (1 % FBS in PBS) for 30 min at 4 °C. Afterwards, cells were washed several times and measured by FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA) flow cytometer. Data were analyzed by using FlowJo software (Tree Star Inc., Ashland, OR, USA). Use of PBMCs for in vitro studies is in accordance with the local ethics committee (approved proposal # 327/14).

Glioblastoma cell lines

Human glioblastoma cell lines, U87-MG (U87) or A172 (American Type Culture Collection, Manassas, VA, USA), were cultured in DMEM supplemented with 10 % FBS and 1 % penicillin (120 mg/ml)/streptomycin (120 mg/ml) (Life Technologies). Cells were incubated with CatG inhibitor I (10 µM), E64d (10 µM), pepstatin A (10 µM), lactacystin (10 µM), or DMSO for 24 h at 37 °C. Cell surface MHC I was determined by flow cytometry as described above.

Active-site label and Western blot

Cells were lysed (PBS pH 7.4 and 0.5 % IGEPAL CA-630), adjusted for equal protein content, and 10 µg of cell lysate was incubated with the activity-based probe Marcin Sienczyk 116 (MARS116, 2 µM) in PBS pH 7.4 for 1 h at room temperature [13]. In order to detect cysteine proteases, 10 µg of cell lysate was incubated with 50 mM citrate pH 5.0, 50 mM DTT, and Doron C. Greenbaum 04 (DCG-04, 10 µM, probe kindly donated by Steven Verhelst, Technische Universität München) [14]. Samples were resolved (12 % SDS-PAGE), blotted and visualized using HRP (Vectastain, Burlingame, CA, USA). For CatD detection, 20 µg of the respective cell extracts was resolved by SDS-PAGE. Immunoblotting was performed using a specific CatD antibody (Calbiochem, Schwalbach, Germany), while anti-β-actin antibody and secondary HRP-conjugated antibodies were obtained from Sigma-Aldrich or GE Healthcare (Little Chalfont, UK).

Expression vectors

The bi-cistronic CMV promoter-based pCGCG expression vector coexpressing *HIV-1 NL4-3 nef* and the enhanced green fluorescent protein (eGFP) under the translational control of the encephalomyocarditis virus internal ribosome entry site element has been described previously [15]. The pCGCG control vector expressing only eGFP (empty vector) contains a nef gene with a mutation in the initiation codon and two premature stop codons at positions 3 and 40 of the open reading frame. A pCGCG vector coexpressing eGFP and human cathepsin G from bicistronic RNAs was generated by retrieving the sequences from the National Center for Biotechnology Information (NCBI) Protein database (NCBI reference sequence: NP_001902.1) and synthesizing the genes by GenScript (Piscataway, NJ, USA). Standard cloning techniques were used to clone the synthesized sequences with flanking XbaI and MluI site into the pCGCG vector. Sequencing of the insert confirmed that no undesired nucleotide changes were present.

Cell culture and transfections

HEK293T (plasmid: 0.25 µg/ml) and U87 (plasmid: 1.50 µg/ml) were transfected by using Lipofectamine LTX reagent (Life Technologies) or TransIT-LT1 (Mirus Bio LLC, Madison, WI, USA), respectively, according to the manufacturer's instructions. Cells were stained for flow cytometry, collected by FACSCalibur or FACSCanto II (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed by FlowJo software (Tree Star Inc.).

Determination of CatG activity via colorimetric substrate

Kinetic measurement of CatG activity was accomplished by adding 0.5 mg/ml of HEK293T- or U87 (1 mg/ml)-derived cell lysate to the colorimetric substrate Suc-Val-Pro-Phe-pNA (200 µM) in PBS (pH 7.4) as previously described [16]. The enzyme assay was performed in duplicates at 37 °C and absorption was determined at 405 nm (absorbance microplate reader, Bio-Rad, Model 550, Hercules, USA).

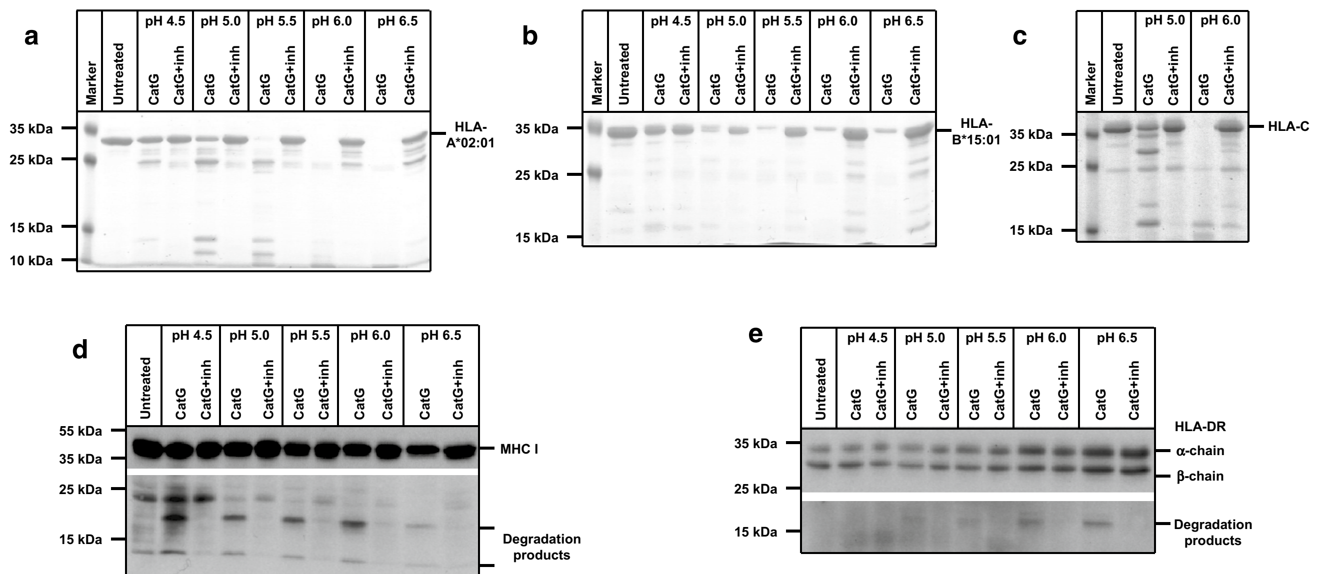


Fig. 1 MHC I molecules are a substrate for CatG. **a**, **b**, and **c** Recombinant soluble HLA-A*02:01, HLA-B*15:01, or HLA-C molecules were incubated with CatG for 2 h at 37 °C. The degradation products were separated by SDS-PAGE and visualized by Coomassie staining. A representative result of three independent experiments is shown. **d** and **e** A B-lymphoblastoid cell line (BLC) was lysed in 0.5 % IGEPAL CA-630, and cell lysate was degraded by CatG for 2 h 37 °C. Matched amounts of total protein were resolved by SDS-PAGE and immunoblotted for MHC I (HC-10) or HLA-DR (CHAMP). Of note, upper panel shorter X-ray film exposure. One of three independent experiments is shown

Sphere-cultured stem cell-enriched glioblastoma cell populations (SCs)

After patient's consent was obtained, astrocytoma grade IV tissues (glioblastoma) were minced, washed in PBS, and incubated with TrypLE Express (Gibco, Life Technologies) for 5 min. Cells were filtered (pore size 70 µm) and cultured in DMEM/F-12 medium (Gibco, Life Technologies,) containing L-glutamine, epidermal growth factor (EGF, Biomol GmbH, Hamburg, Germany), fibroblast growth factor (FGF, Miltenyi Biotec, Bergisch Gladbach, Germany), B27 (Gibco, Life Technologies), 1 % penicillin (120 mg/ml)/streptomycin (120 mg/ml) (Life Technologies) [17]. These cells are herein determined as a sphere-cultured stem cell-enriched glioblastoma cell populations (SCs). In order to differentiate SCs to adherent cells (PCs), PCs were cultured in DMEM supplemented with 10 % FBS plus 2 mM glutamine, 1 % penicillin (120 mg/ml)/streptomycin (120 mg/ml) (Life Technologies). SC (plasmid: 1.5 µg/ml) were transfected by using TransIT-LT1 (Mirus Bio LLC). Cells were stained for flow cytometry as described before and analyzed by FACSCalibur (BD Biosciences). Use of SC is in accordance with the local ethics committee (approved proposal # 162/10).

Statistical analysis

Data were depicted as mean ± standard error of the mean (S.E.M.), and statistical analysis was performed using the unpaired, two-tailed Student's *t* test (Prism 4, GraphPad Software, La Jolla, CA, USA).

Results and Discussion

CatG, CatS, and CatD proteolytically degrade MHC I molecules in vitro

In antigen presenting cells, cell surface MHC I molecules are either recycled to present a new set of endocytic-derived antigenic peptides or directed to lysosomes for degradation [3, 18–20]. However, proteases mediating this MHC I turnover need to be identified. Therefore, recombinant MHC I molecules were incubated with a panel of different human proteases (cathepsins). Cathepsin G (CatG) progressively processed three different human MHC I loci (HLA-A*02:01, HLA-B*15:01, and HLA-C) under various pH conditions (pH 4.5–6.5) with its highest processing rate at pH 6.5 (Fig. 1a–c). To analyze whether cell-derived MHC molecules are a natural substrate for CatG, cell lysate from a non CatG-expressing B cell line was incubated with CatG at a pH ranging from 4.5 to 6.5. MHC I and MHC II-specific immunoblot analysis showed that MHC I was digested at all indicated pH conditions (Fig. 1d, e, and Supplementary Figure 1, left panel), while MHC II molecules were only cleaved at a higher pH as we previously published [21]. In a further set of experiments, we investigated whether the

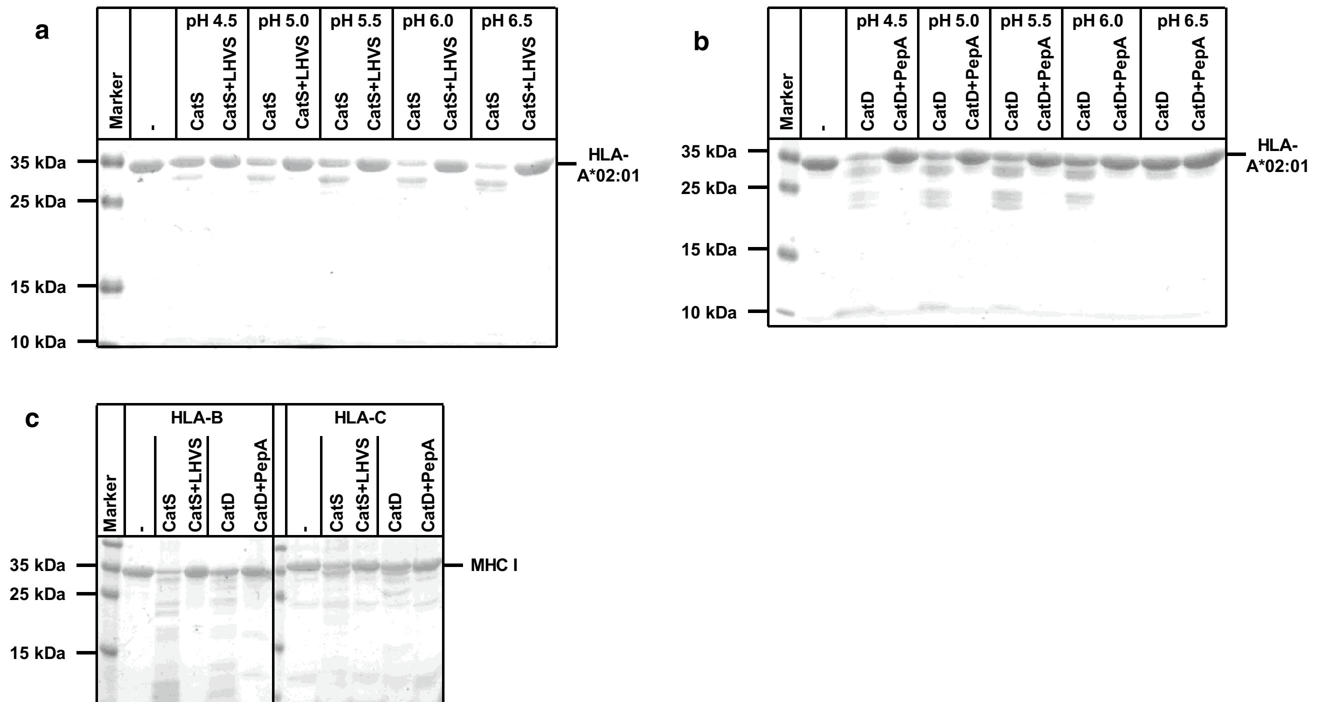


Fig. 2 CatS and CatD degrade MHC I molecules. **a** and **b** Recombinant soluble HLA-A*02:01 molecules were incubated with CatS or CatD, respectively, for 2 h at 37 °C at different pHs. The degradation products were separated by SDS-PAGE and visualized by Coomassie staining. Three independent experiments were performed. **c** HLA-B*15:01 and HLA-C were incubated with CatS at pH 6.0 or CatD at pH 5.0. Two independent experiments were performed

cysteine protease CatS or the aspartic protease CatD hydrolyzes HLA-A*02:01. Both CatS (pH 4.5–6.5) and CatD (pH 4.5–5.5) degraded HLA-A*02:01, HLA-B*15:01, and HLA-C molecules in a pH-dependent manner (Fig. 2a–c).

Next, we assessed whether membrane embedded MHC I molecules are accessible for degradation by CatG. To this end, a B cell line was incubated with purified CatG and the resulting peptide degradation pattern in the B cell line supernatant was analyzed by mass spectrometry. We found several MHC I-, but no MHC II-derived fragments (Supplementary Figure 1, right panel). Our results confirm that MHC II molecules are protected from proteolytic degradation if tethered to the membrane [21]. This is in stark contrast to MHC I molecules which are efficiently processed by CatG even when associated with membranes.

MHC I molecules are increased by CatG inhibition

Further experiments were performed to address the question whether the density of cell surface MHC I molecules is

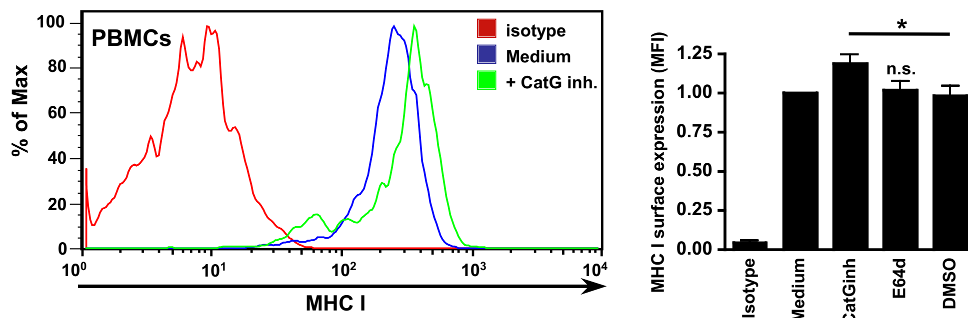


Fig. 3 Cell surface MHC I is increased by CatG inhibition. PBMCs were cultured in the presence or absence of the cell permeable CatG inhibitor (10 µM), cysteine protease inhibitor (E64d, 10 µM), or vehicle control (DMSO) for 24 h and expression of cell surface MHC I was analyzed by flow cytometry (gated for a monocyte population, *left panel*). Five independent experiments were normalized to the medium control and summarized in a *bar diagram* (*right panel*, PBMCs from five different donors, $n = 5$)

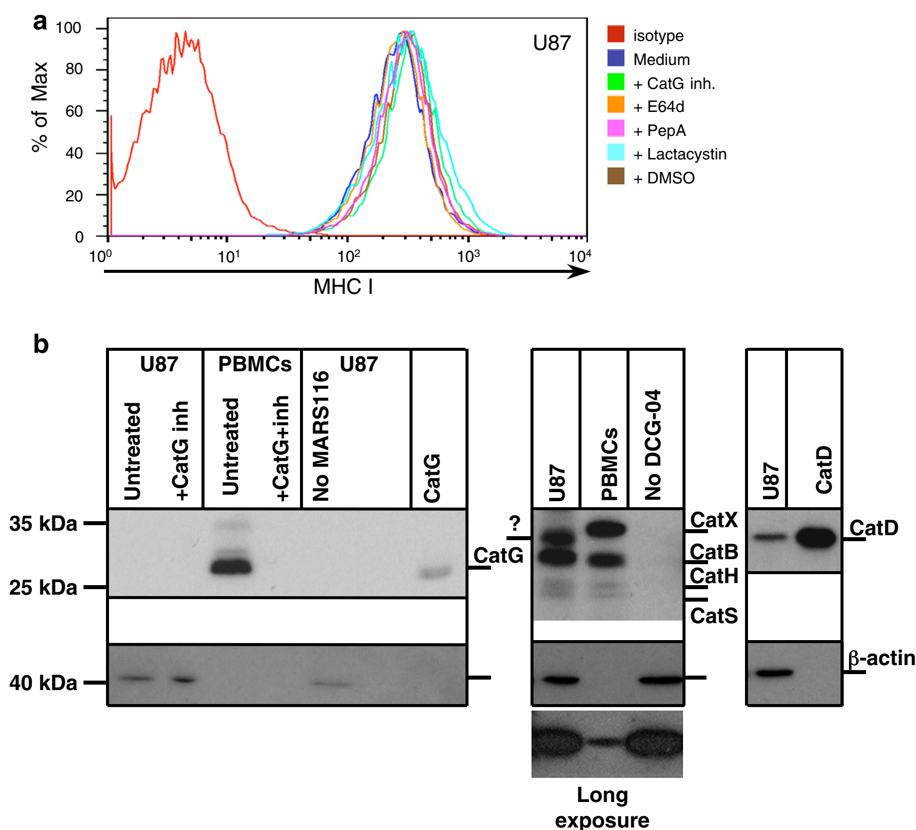


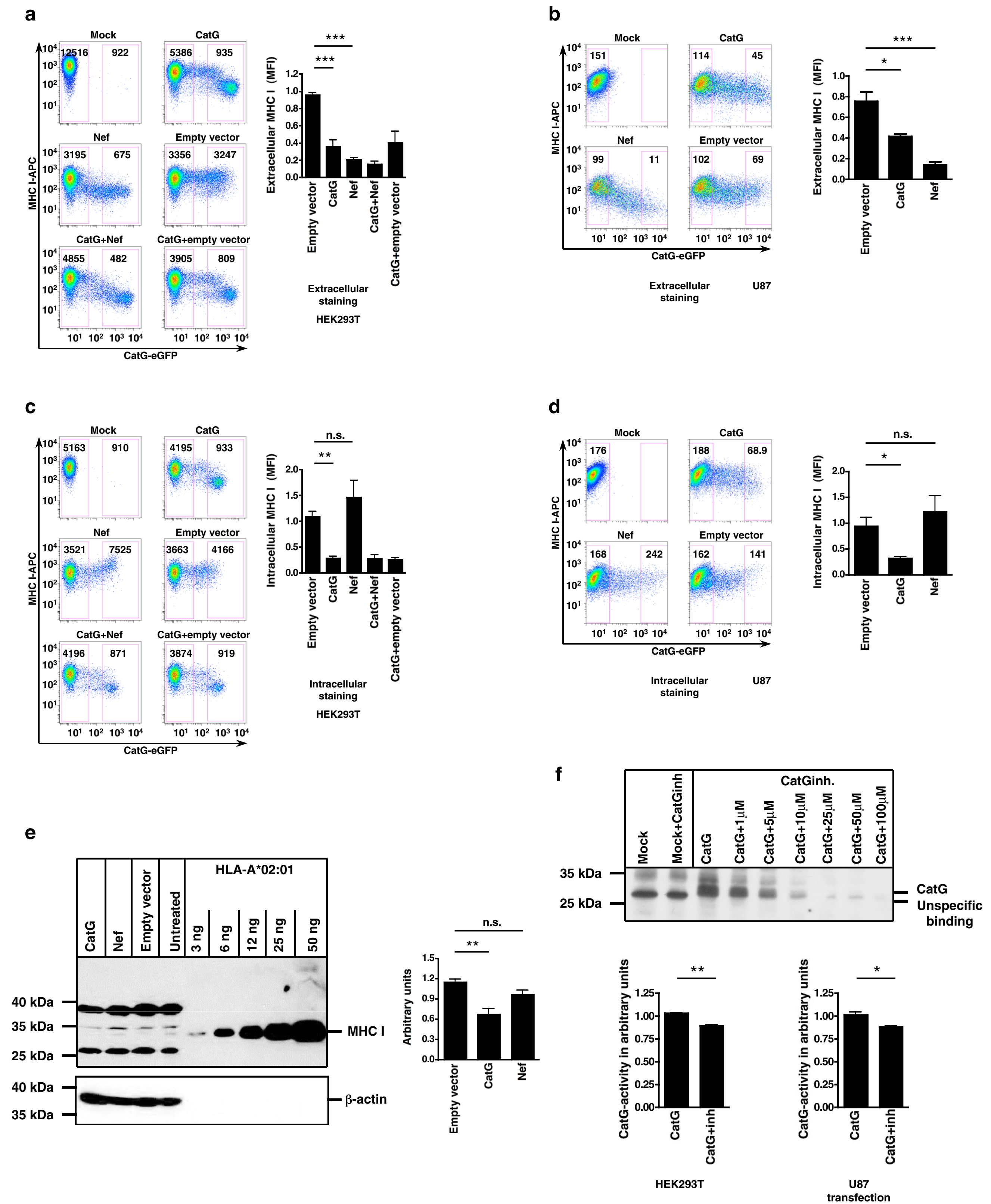
Fig. 4 U87 cells do not exhibit CatG activity. **a** The human glioblastoma cell line, U87, was cultured in the presence or absence of the cell permeable CatG inhibitor (10 μM), cysteine protease inhibitor (E64d, 10 μM), aspartic protease inhibitor, pepstatin A (PepA, 10 μM), proteasome inhibitor (lactacystin, 10 μM), or vehicle control (DMSO) for 24 h, and expression of cell surface MHC I was analyzed by flow cytometry (*left panel*). A representative set out of three independent experiments is shown. **b** In order to determine cysteine or serine protease activity, cell lysate was incubated with the activity-based probe MARS116 (*left panel*) or DCG-04 (*middle panel*). Both activity-based probes form a covalent bond to the active center of protease which can be resolved by SDS-PAGE and visualized via streptavidin-HRP blot. The same samples were pre-incubated with the CatG inhibitor (100 μM). Cell lysate from U87 or PBMCs was resolved by SDS-PAGE, and immunoblot was performed with anti-CatD (*right panel*). β-actin served as a loading control. Two independent experiments were performed (for CatG activity $n = 3$)

regulated by CatG in situ. Peripheral blood mononuclear cells (PBMCs) were treated with either the cell permeable CatG inhibitor or cysteine protease inhibitor E64d. While PBMCs treated with the CatG inhibitor showed an increase of cell surface MHC I (Fig. 3 and Supplementary Figure 2). In cells treated with E64d, levels of MHC I were not significantly changed. Taken together, these results show that it is feasible to indirectly manipulate MHC I surface expression by applying a CatG inhibitor but not by blocking cysteine proteases.

Overexpression of CatG in HEK293T and U87 reduces cell surface MHC I

To survive recognition by CTLs, tumor cells reduce MHC I transcription to avoid newly synthesized MHC I molecules presenting tumor-associated antigens (peptides) on the cell surface [6]. Nevertheless, some cell surface MHC I molecules are necessary to preserve tumor cells from elimination by NK cells, since these cells are activated by the absence of MHC I [22]. To elucidate the involvement of proteases in regulating MHC I in non-immune cells, two human glioblastoma cell lines (U87 and A172),

Fig. 5 Overexpression of CatG in HEK293T and U87 cells. **a** Transfection of HEK293T or **b** U87 with CatG or Nef was controlled by green fluorescence and levels of cell surface MHC I were quantified by flow cytometry. Empty vector served as a negative control. One representative set out of at least four independent experiments is shown (HEK293T, $n = 4$; U87, $n = 5$) independent experiments is shown, *left panel* and the summary, *right panel*. **c** In a similar setting to above, intracellular staining was performed to detect levels of MHC I by flow cytometry in HEK293T cells. One representative experiment is shown (*left panel*) and the summary of $n = 4$ (*right panel*). **d** Intracellular staining of MHC I for U87 cells. One representative experiment is shown, *left panel* and the summary of $n = 3$, *right panel*. **e** Detection of total MHC I molecules in HEK293T cells by HLA-A, HLA-B, and HLA-C immunoblot. One representative experiment is shown, *left panel* and the summary of $n = 3$, *right panel*. **f** CatG-transfected HEK293T (*left panel*) or U87 cells (*right panel*) were analyzed for CatG activity by using the activity-based probe MARS116 or the colorimetric substrate Suc-VPF-pNA. Two independent experiments were performed ▶



sphere-cultured stem cell-enriched cell populations (SCs), and FBS-differentiated SCs (PCs) from three different glioblastoma patients were incubated with the indicated cell permeable inhibitors and levels of cell surface MHC I molecules were analyzed. No differences in MHC I cell surface expression were observed when applying the

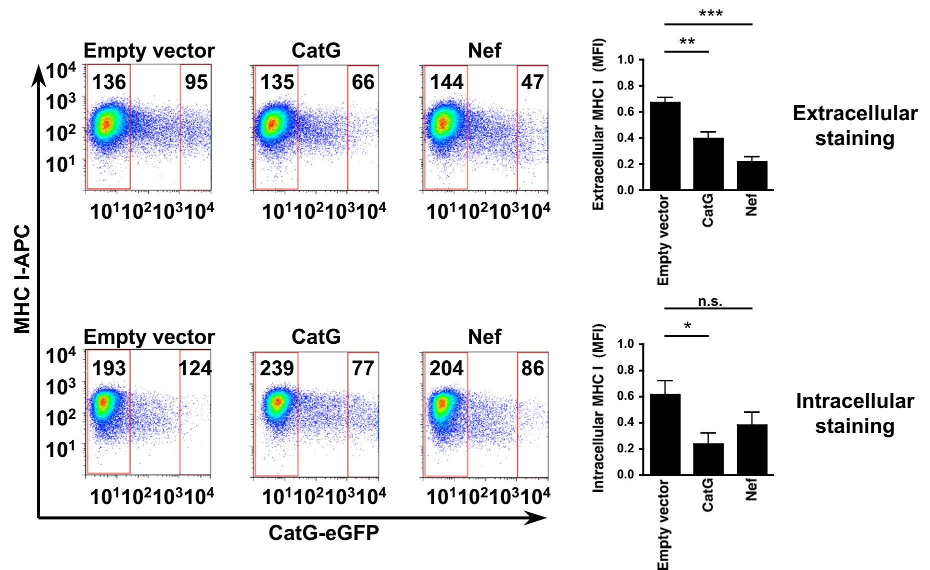


Fig. 6 CatG overexpression in a sphere-cultured stem cell-enriched glioblastoma cell populations (SCs). Transfection of SC35 with CatG, Nef, or empty vector was controlled by green fluorescence, and levels of cell surface MHC I were quantified by flow cytometry. One representative set out of four independent experiments is shown, *left panel* and the summary, *right panel*. All values were normalized to the sample transfected with the control eGFP plasmid

CatG inhibitor (Fig. 4a, Supplementary Figures 3 and 4), since CatG activity was absent in all glioblastoma cell populations tested (Fig. 4b, left panel and Supplementary Figure 5). Although we did not find any differences in cell surface expression of MHC I when glioblastoma cell lines were treated with cysteine or aspartic protease inhibitors, U87 cells did feature active CatB and, to a lesser extent, CatS-activity, which was detected by the activity-based probe DCG-04 (Fig. 4b, middle panel). Furthermore, a CatD-specific immunoblot revealed that U87 expressed a robust level of this protease (Fig. 4b, right panel). A summary of CatB, CatD, CatS, and CatX expression in A172, T98, U87, PC35, PC38, PC40, SC35, SC38, and SC40 is shown in the Supplementary Figure 6. These data expand on previous literature which showed CatB, CatD, and CatS expression in glioblastoma cell lines (summarized in [23]) but did not analyze PCs and SCs.

The finding that CatG was absent in glioblastoma cells raised the question whether ectopic expression of CatG might downregulate MHC I in these cells. Thus, we transfected CatG-negative HEK293T and U87 cells with a plasmid containing the CatG gene and a reporter gene, enhanced green fluorescence protein (eGFP). Transfection (eGFP) and levels of MHC I (allophycocyanin) were simultaneously quantified by flow cytometry. Expression of CatG drastically reduced MHC I cell surface levels in both HEK293T and U87 (Fig. 5a, b) compared to the empty vector control. Furthermore, HEK293T and U87 were transfected with the human immunodeficiency virus (HIV) nef protein, since a previous publication demonstrated reduced levels of cell surface MHC I upon Nef expression in U937 and T cells [24]. Total amounts of MHC I molecules were detected by intracellular staining or immunoblot showing a reduction of MHC I for CatG-transfected HEK293T, U87, A172, and three additional glioblastoma cell lines (Fig. 5c–e and Supplementary Figure 7), and CatG activity in CatG-transfected HEK293T and U87 was independently confirmed by active-site label and the colorimetric substrate assay (Fig. 5f). This is in contrast to Nef-transfected HEK293T and U87 which exhibited an accumulation of MHC I, most likely due to the lack of CatG in these cells. This dataset demonstrates that CatG plays a pivotal role for MHC I regulation, and its overexpression is sufficient to degrade MHC I and thus downregulate cell surface MHC I in glioblastoma cells.

CatG proteolytically downregulates levels of MHC I in glioblastoma stem cells

Having verified CatG-mediated MHC I degradation in different glioblastoma cell lines, we sought to determine whether CatG has also immunomodulatory properties in downregulating MHC I in glioblastoma stem cells. For this purpose, sphere-cultured stem cell-enriched cell populations (SCs) from three different glioblastoma patients, either transfected with CatG, Nef, or empty vector, were analyzed for MHC I. We observed that SCs, which did not express endogenous CatG, significantly degraded both extra- and intracellular MHC I upon CatG-transfection, while Nef-transfected SCs only demonstrated reduced extracellular MHC I compared to empty vector-transfected SCs (Fig. 6 and Supplementary Figure 8).

In conclusion, we demonstrate that CatG degrades MHC I molecules suggesting that CatG is an essential protease for post-transcriptional regulation of MHC I molecules. Strikingly, glioblastoma cells do not harbor active CatG. We speculate that these cells (1) lost their ability

to proteolytically degrade MHC I by CatG during tumorigenesis to, (2) prevent complete reduction of cell surface MHC I, and thus (3) avoid NK cell activation. Therefore, restoration of CatG in glioblastoma presents an opportunity to sensitize glioblastoma cells to NK-mediated killing by completely removing MHC I from the cell surface.

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Compliance with ethical standards

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

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