Neuroscience Letters 660 (2017) 109–114

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

ADAM17 is the main sheddase for the generation of human triggering receptor expressed in myeloid cells (hTREM2) ectodomain and cleaves TREM2 after Histidine 157

Dominik Feuerbach[a,*], Patrick Schindler[b], Carmen Barske[a], Stefanie Joller[a], Edwige Beng-Louka[b], Katie A. Worringer[c], Sravya Kommineni[c], Ajamete Kaykas[c], Daniel J. Ho[c], Chaoyang Ye[c], Karl Welzenbach[d], Gaelle Elain[d], Laurent Klein[d], Irena Brzak[a], Anis K. Mir[d], Christopher J. Farady[d], Reiner Aichholz[e], Simone Popp[b], Nathalie George[b], Ulf Neumann[a]

[a] Neuroscience Research, Novartis Institutes for Biomedical Research, Basel, Switzerland
[b] Biologics Center, Novartis Institutes for Biomedical Research, Basel, Switzerland
[c] Neuroscience Research, Novartis Institutes for Biomedical Research, Cambridge, MA, USA
[d] Autoimmunity, Transplantation & Inflammation, Novartis Institutes for Biomedical Research, Basel, Switzerland
[e] PK Sciences Department, Novartis Institutes for Biomedical Research, Basel, Switzerland

ARTICLE INFO

Keywords:
Shedding
Alzheimers disease
Macrophages
Neuroinflammation

ABSTRACT

Triggering receptor expressed in myeloid cells (TREM2) is a member of the immunoglobulin superfamily and is expressed in macrophages, dendritic cells, microglia, and osteoclasts. TREM2 plays a role in phagocytosis, regulates release of cytokine, contributes to microglia maintenance, and its ectodomain is shed from the cell surface. Here, the question was addressed at which position sheddases cleave TREM2 and what are the proteases involved in this process. Using both pharmacological and genetic approaches we report that the main protease contributing to the release of TREM2 ectodomain is ADAM17, (a disintegrin and metalloproteinase domain containing protein, also called TACE, TNFα converting enzyme) while ADAM10 plays a minor role. Complementary biochemical experiments reveal that cleavage occurs between histidine 157 and serine 158. Shedding is not altered for the R47H-mutated TREM2 protein that confers an increased risk for the development of Alzheimers disease. These findings reveal a link between shedding of TREM2 and its regulation during inflammatory conditions or chronic neurodegenerative disease like AD in which activity or expression of sheddases might be altered.

1. Introduction

Triggering receptor expressed in myeloid cells (TREM2) is a type I transmembrane glycoprotein and a member of the immunoglobulin (Ig) receptor superfamily [3]. TREM2 expression has been shown in macrophages, dendritic cells, microglia and osteoclasts [16,20,21], and expression seems to be temporally and spatially regulated. In macrophages expression is upregulated during the course of inflammation, e.g. expression peaks 2–3 days after thioglycolate challenge in a murine model of peritonitis [25]. TREM2 is also enriched at those microglia cell surface regions which contact Aβ plaques or neuronal debris [30]. Some of the ligands that are sensed by TREM2 in this environment have recently been identified, for example phospholipids and myelin lipids [18] as well as ApoE [1,2]. Other ligands could be Aβ and plaque associated neuronal debris since TREM2 contributes to the uptake of Aβ into microglia [29].

TREM2 can be shed from the cell surface and the shed ectodomain of TREM2 (sTREM2) in human CSF has been assessed as a potential AD biomarker and has been shown to be increased during ageing in general [23]. Detailed analysis during the course of AD revealed that sTREM2 increases early in AD before clinical symptoms appear, peaks in MCI-AD, and stays elevated but at lower levels compared to the MCI-AD stage in AD dementia [23].

sTREM2 comprises the IGSF domain and part of the stalk region, but the exact size is a matter of debate. The entire TREM2 protein consists of a leading signal peptide (amino acids 1–18), a single V-type IgSF

Abbreviations: TREM2, Triggering receptor expressed on myeloid cells; AA, amino acids; DAP12, DNAX activating protein 12; ADAM1, a disintegrin and metalloproteinase domain containing protein; TACE, TNFα converting enzyme

* Corresponding author.
E-mail address: dominik.feuerbach@novartis.com (D. Feuerbach).

http://dx.doi.org/10.1016/j.neulet.2017.09.034
Received 4 July 2017; Received in revised form 22 August 2017; Accepted 14 September 2017
Available online 18 September 2017

extracellular region, (amino acids 19–132), a stalk region (amino acids 133–172), a positively charged transmembrane domain (amino acids 173–197), and a cytosolic tail (amino acids 198–230) [11,12]. In spite of the long cytosolic tail, there is no signaling motif. Instead, TREM2 forms a heterodimer with DAP12 and the lysine within the transmembrane region of DAP12 interacts with aspartic acid in the transmembrane part of TREM2 [3]. Shedding of TREM2 is thought to involve as first step ADAM10 (a disintegrin and metalloproteinase domain containing protein) and/or ADAM17 (also termed TACE, TNFα converting enzyme) [10]. This proteolytic cleavage liberates sTREM2 from the plasma membrane. Next, γ-secretase cuts the membrane associated C-terminal fragment (CTF) enabling further degradation of the peptide [28]. Inhibition of γ-secretase results in accumulation of CTF and trapping of DAP12 within this complex. This leads to reduced TREM2 signaling as availability of DAP12 becomes limiting.

In the current study we have investigated the differential contribution of ADAM10 and ADAM17 to shedding of TREM2 ectodomain using a pharmacological and a genetic approach. Next we identified the cleavage site of ADAM10/ADAM17 within the stalk region of TREM2 using complementary biochemical approaches.

2. Material and methods

2.1. Compounds

GI254023 ((2R,3S)-3-(formyl-hydroxyamino)-2-(3-phenyl-1-propyl) butanoic acid [(1S)-2,2-dimethyl-1-methylcarbamoyl-1-propyl] amide) was synthesized as described in [9]). DPC333 ((2R)-2-((3R)-3-amino-3{4-[2-methyl-4-quinolinyl) methoxy]

phenyl}-2-oxopyrrolidinyl)-N-hydroxy-4-methylpentanamide)) was synthesized as described in [19].

2.2. Cell culture

THP1 cells stably co-expressing Cas9 and a blasticidin resistance gene delivered by lentivirus were cultured in RPMI medium containing 10% FBS, 1% L-glutamine, 1% pen/strep, and 10 μg/ml of blasticidin (Thermo Fisher Scientific). The cells were cultured at 37 °C in 5% CO2 atmosphere.

CHO cells were transfected to co-express hDAP12 with a hygromycine resistance gene and hTREM2 with a neomycine resistance gene driven by the CMV promotor using Lipofectamine LTX reagent (Thermofischer) according to the manufacturer's recommendations. One positive clone was selected and designated CHO-hDAP12-hTREM2. Cell lines were used for experiments up to a maximal passage number of 35.

Human M2A macrophages were obtained from buffy coat using a negative isolation kit for monocytes (Stem cell technologies) and differentiated for 5 days.

2.3. Live cell imaging

Human M2A macrophages or CHO-hDAP12-hTREM2 were seeded on 384 well plates (Greiner) and treated with ADAM inhibitors DPC333 or GI254023 at concentrations indicated in Figs. 16 h later cells were treated for 30 min with PMA (50 ng/ml) or 0.1% DMSO. Plates were put on ice and stained with goat anti human TREM2 antibody AF1828 (R & D Systems, 3 μg/ml) or isotype control and Hoechst stain followed by incubation with the secondary Alexa Fluor 488 conjugated anti goat antibody (Molecular Probes). Images were acquired using an InCell2000 analyzer (GE Healthcare). For image quantification the free open source software CellProfiler was applied.

2.4. Immuno-purification

Shed TREM2 was purified from cell supernatant (conditioned medium collected from intact cells) through microscale immuno-purification. This was performed on the MEA platform using Streptavidin coated tips (PTR 92-05-05, Phynexus). Tips were equilibrated with PBS, then 200 μL of biotinylated anti-TREM2 antibody (0.55 pmol/μL, BAF1828 from R & D System) was loaded onto the streptavidin μcolumn (5 μL bed volume) at a speed of 0.25 mL/min and 8 passages. After a wash with PBS, shed TREM2 is captured from the cell supernatant (200 μL) at a speed of 25 mL/min and 12 passages. It is followed by PBS wash and elution by 0.1 M glycine pH 2.5 for a final volume of 2 × 60 μL. The latter solution is neutralized with the addition of 5 μL of 1 M Tris-HCl pH 10, then it was dried using a Speedvac and rehydrated with 8 M urea (5 μL, Fluka) and 0.4 M NH_4CO_3 (30 μL, Fluka). The sample is then reduced (2 μL of 1 M DTT, 30 min at 50 °C), alkylated (6 μL of 1 M IAA (Sigma), 30 min at RT in dark) and the reaction was terminated with the addition of 1 M DTT (2 μL) and 0.4 M NH_4CO_3 (30 μL). The resulting sample is either digested overnight by Trypsin (Promega) or Asp-/Glu-C enzyme (Roche). The digested sample is finally acidified with HCOOH (1 μL, Fluka) and 25 μL of the resulting digest is injected onto the LC–MS platform.

2.5. Statistical analysis

Statistical analysis was performed using Prism software (GraphPad, San Diego, CA) using ANOVA and student's *t*-test where appropriate. A p value of < 0.05 was considered significant.

3. Results

3.1. ADAM17 inhibitors stabilize TREM2 at the cell surface

To determine the contribution of ADAM10 or ADAM17 to shedding of TREM2 ectodomain we first set out to find selective inhibitors and identified two compounds (DPC333 [19] and GI254023 [9]) that were characterized for inhibitory selectivity towards ADAM10 and ADAM17 (see supplemental Fig. 1). While DPC333 is a more potent inhibitor on ADAM17 ($IC_{50} < 0.6$ nM) than on ADAM10 ($IC_{50} = 5.3$ nM), GI254023 displays selectivity for ADAM10 (IC_{50} 1.5 nM) over ADAM17 ($IC_{50} = 196$ nM). Using live cell imaging, cell surface expression of hTREM2 was assessed in CHO-hDAP12-hTREM2 cells after overnight treatment of the cells with the two ADAM inhibitors under conditional shedding conditions (Fig. 1A) or after treatment of the cells with PMA (Fig. 1B). The ADAM17 selective inhibitor DPC333 dose-dependently increases TREM2 cell surface levels under both conditions. A limited effect on TREM2 cell surface levels is also observed at higher concentrations for GI254023, but only under steady state conditions. This effect might be attributed to true ADAM10 inhibition or it might be caused by unspecific inhibition of ADAM17 by GI254023 when used at high concentrations. In PMA-treated cells there is a complete lack of effect of GI254023 on TREM2 cell surface expression (Fig. 1B). To get closer to a physiological cellular system, a similar experiment was conducted in human M2A macrophages differentiated from $CD14^+$ human monocytes (Fig. 1C and D). These results replicate very well the initial findings in CHO-hDAP12-hTREM2 cells; the ADAM17 inhibitor DPC333 increases TREM2 cell surface expression dose-dependently under both conditions (Fig. 1C and D) and the selective ADAM10 inhibitor displays a small effect on steady-state shedding (Fig. 1C). In summary, these experiments indicate that in human macrophages, ADAM17 plays a critical role for TREM2 shedding but a marginal contribution of ADAM10 under steady state condition cannot be excluded.

3.2. ADAM17 ablation in THP1 cells reduces constitutive shedding

To confirm these conclusions, a genetic approach was used to further investigate the contribution of ADAM10/17 to TREM2 shedding. Human monocytic THP1 cells were chosen as model system which