Clinical Immunology (2015) 160, 172–179

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

BRIEF COMMUNICATION

Fluorescent tags influence the enzymatic activity and subcellular localization of procaspase-1

Michael C. Heymann [1], Sabrina Rabe [1], Susanne Ruß, Franz Kapplusch, Felix Schulze, Robert Stein, Stefan Winkler, Christian M. Hedrich, Angela Rösen-Wolff, Sigrun R. Hofmann*

Department of Pediatrics, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

Received 22 October 2014; accepted with revision 14 May 2015
Available online 27 May 2015

KEYWORDS
Caspase-1;
CARD;
Fluorescent fusion protein;
mCherry;
Inflammasome;
IL-1β

Abstract Subcellular localization studies and life cell imaging approaches usually benefit from fusion-reporter proteins, such as enhanced green fluorescent protein (EGFP) and mCherry to the proteins of interest. However, such manipulations have several risks, including protein misfolding, altered protein shuttling, or functional impairment when compared to the wild-type proteins. Here, we demonstrate altered subcellular distribution and function of the pro-inflammatory enzyme procaspase-1 as a result of fusion with the reporter protein mCherry. Our observations are of central importance to further investigations of subcellular behavior and possible protein–protein interactions of naturally occurring genetic variants of human procaspase-1 which have recently been linked to autoinflammatory disorders.

1. Introduction

The proinflammatory enzyme caspase-1 which plays a central role in the innate immune system, is involved in a variety of inflammatory conditions [1], and has recently been linked to rare autoinflammatory diseases [2]. In a cohort of patients with recurrent fevers and systemic inflammation, who did not carry mutations in previously reported fever genes (including NLRP3, MEFV, MVK, PSTPIP1), variants in the *CASP1* gene (encoding for procaspase-1) were detected [2]. Interestingly, these variants caused impairment or complete loss of caspase-1 activity, resulting in markedly diminished or abolished IL-1β activation and release and a trend to elevated IL-6 and TNF-α secretion [2]. Though reduced IL-1β expression and systemic inflammation appear to be contradicting observations, most recent findings indicate that the aforementioned naturally occurring procaspase-1 variants mediate increased NF-κB activation through their interaction with receptor interacting protein kinase 2 (RIP2) [3].

* Corresponding author at: Dept. of Pediatrics, University Hospital Carl Gustav Carus, Fetscherstrasse 74, 01307 Dresden, Germany. Fax: +49 351 458 4381.
E-mail address: sigrun.hofmann@uniklinikum-dresden.de (S.R. Hofmann).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.clim.2015.05.011
1521-6616/

Our current understanding of caspase-1 activation comprises danger signals and pattern recognition receptors, mediating inflammasome assembly, subsequent activation of caspase-1, and cleavage of IL-1β and IL-18 precursors into their active forms [4,5].

Activated cytokines are then released from monocytes and macrophages, inducing down-stream immunological answer to the aforementioned danger signals [6]. Prior to its activation, caspase-1 is present in the cytosol, and referred to as procaspase-1. Procaspase-1 structurally consists of three domains: i) an N-terminal caspase recruitment domain (CARD), which is important for the activation of procaspase-1 mediated nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) [7,8], ii) a central p20 domain and iii) a C-terminal p10 domain [9,10]. Usually, direct proximity of procaspase-1 molecules results in activation of caspase-1 by its auto-processing into the p10 and p20 subunits [11]. The assembly of two p10 and two p20 subunits to a hetero-tetrameric molecule results in active caspase-1 that is responsible for cleaving pro-IL-1ß and pro-IL-18 into their mature and active forms IL-1ß and IL-18 respectively.

In order to trace subcellular protein trafficking, aiming to discover underlying molecular events mediated by the aforementioned *CASP1* variants, we fused the procaspase-1 encoding gene *CASP1* to the genes encoding for the fluorophores mCherry or enhanced green fluorescent protein (EGFP). In combination with modern microscopy and laser-based methods, such as Fluorescence-lifetime imaging microscopy (FLIM), Fluorescence Resonance Energy Transfer (FRET), Fluorescence cross correlation spectroscopy (FCCS), and super-resolution optical imaging methods (Stimulated Emission Depletion, STED; structured illumination microscopy, SIM; Stochastic optical reconstruction microscopy, STORM), further protein attributes such as structure, size, shape and mobility can be addressed.

Here we demonstrate, that fusion of procaspase-1 to the reporter protein mCherry critically influences the enzymatic activity, NF-κB activation capacity, and the subcellular localization of procaspase-1. Alterations largely depended on the position of the reporter protein either at the N-terminal or C-terminal residues of procaspase-1.

2. Materials and methods

2.1. Plasmids

The wild-type procaspase-1 gene or the inactive p.C285A variant, both fused to a flag-tag at their C-terminus were introduced into the lentiviral transfer vector pRRL.SIN.cPPT.SFFV.GFP.WPRE (kindly provided by Prof. C. Baum, Institute of Experimental Hematology, Hannover Medical School, Germany [12]). Wild-type procaspase-1 and the p.C285A variant were introduced into the lentiviral transfer vector p6NST51 when fused to mCherry at their N-terminus (N-pcasp1), or into the lentiviral transfer vector p6NST53 when fused to mCherry at their C-terminus (pcasp1-C). Both the p6NST51 and p6NST53 vectors were kind gifts from Prof. Dirk Lindemann, Institute of Virology, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany [3,13]. The plasmids only differ in their resistance for eukaryotic selection. *mCherry* was purchased from BD Clontech (Mountain View, CA). The pro-IL-1β expression plasmid on a pCR3 backbone was a kind gift from Prof. Jürg Tschopp, Faculté de Biologie et de Médecine, Department of Biochemistry, University of Lausanne, Switzerland. The luciferase reporter plasmid pBVIx-Luc was a kind gift from Prof. Gabriel Nunez, University of Michigan, Ann Arbor, MI. The luciferase reporter plasmid Rluc pRLTK was kindly provided by Prof. Hans Schackert, Department of Surgical Research, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany.

2.2. Antibodies

The following antibodies were used: anti-procaspase-1 A-19 (CARD domain; sc-622), anti-caspase-1 C-20 (p10 domain; sc-515), and anti-IL-1β H-153 (sc-7884), all from Santa Cruz Biotechnology (Santa Cruz, CA); anti-β-actin AC-74 (A5316) from Sigma (Saint Louis, MO); horseradish peroxidase (HRP)-linked anti-rabbit (NA9340) from GE Healthcare (Freiburg, Germany); HRP-linked anti-mouse (P0260) from DakoCytomation (Glostrup, Denmark); and Alexa Fluor 488-linked anti-rabbit (A-11008) from Life Technologies (Darmstadt, Germany).

2.3. Cell culture and transfection

We used Human Embryonic Kidney 293T (HEK 293T) (ATCC, LGC Standards GmbH, Wesel, Germany) cells, which do not express endogenous procaspase-1 or pro-IL-1ß. Cells were cultured in DMEM 24 h prior transfection with the respective plasmids using 1.75 μg/ml polyethylenimine (PEI) from Sigma (Saint Louis, MO), and evaluated 24 h later.

2.4. Measurement of extracellular IL-1β

Concentrations of mature IL-1β in the supernatant were determined using cytometric bead arrays (CBA) from Becton Dickinson (Franklin Lakes, NJ).

2.5. Analysis of protein patterns

Cells were lysed and processed as reported previously [3,14]. Cell lysates were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), subjected to western blotting, and immunochemical detection using the respective antibodies.

2.6. NF-κB luciferase reporter assay

Relative NF-κB activity was determined using the Dual-Glo® Reporter Assay System from Promega (Mannheim, Germany), according to the manufacturer's protocol. HEK 293T cells were transfected with the indicated plasmids including pBXIVluci (carrying a firefly luciferase cDNA driven by 5× NF-κB-binding sites, Stratagene, La Jolla, CA) and of pRL-TK (carrying renilla luciferase cDNA driven by the HSV-Thymidine kinase promoter, Promega, Madison, WI). The total amount of DNA in each transfection was kept constant by the addition of corresponding empty vector of the respective *CASP1* encoding expression plasmid. After 24 h, cell lysates were prepared and the firefly and renilla luciferase activities were measured sequentially on