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STIM1 is cleaved by calpain

Daniel Prins, Marek Michalak *

Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2S7, Canada

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ABSTRACT

Store-operated calcium entry (SOCE) is a pathway that moves Ca^{2+} across the plasma membrane and is mediated by two major proteins, STIM1 and Orai1. Here, we discovered that the cytoplasmic domain of STIM1 is a target for calpains, a family of Ca^{2+}-activated proteases. We found that calpain cleavage of STIM1 serves to control its cellular abundance and was noticeably increased under conditions of cellular stress and apoptosis. Dysregulation of STIM1 levels has been reported to have human disease consequences and our results suggest a mechanism for controlling STIM1 abundance.

1. Introduction

Ca^{2+} ions serve as versatile intracellular messengers; as such, the cell has evolved various pathways to properly control the movement of Ca^{2+} ions across various membranes and the accumulation of ions within cellular organelles. One such pathway is store-operated calcium entry (SOCE), which couples depletion of ER luminal Ca^{2+} stores with Ca^{2+} influx across the plasma membrane [1]. SOCE is mediated by two key proteins: stromal interaction molecule 1 (STIM1), an ER transmembrane protein serving as a Ca^{2+} store sensor [2,3]; and calcium release-activated calcium channel (Orai) family membrane proteins, which serve as plasma membrane Ca^{2+} channels [4]. Structurally, STIM1 can be separated into several domains: a luminal domain, containing a Ca^{2+}-binding EF hand and a sterile-α-motif (SAM) [5]; a transmembrane domain; and a cytoplasmic domain, containing several coiled-coil domains, regions rich in serines and prolines, and a region rich in lysines [6]. Initiation of SOCE occurs via Ca^{2+} dissociation from the luminal domain of STIM1, leading to homooligomerization of STIM1 [7]. The cytoplasmic coiled-coil domains of STIM1 are thought to further mediate the oligomerization of STIM1 as well as mediate direct contact between STIM1 and Orai1 [8]. Additionally, the cytoplasmic domain is implicated in controlling the stability and turnover of STIM1; STIM1 lacking its cytoplasmic domain is noticeably less stable than is wild-type STIM1 [9]. STIM1 and SOCE have been implicated in several different disease states: non-functional STIM1 is associated with severe combined immunodeficiency [10], while increased STIM1 levels and SOCE have been linked to increased metastatic behavior in breast cancer [11].

Calpains are a family of Ca^{2+}-sensitive, neutral pH proteases; there are fourteen known isoforms in humans, with the best-characterized and most ubiquitously expressed being CAPN1 and CAPN2 [12]. These both exist as heterodimers, consisting of a large catalytic subunit and a small regulatory subunit, CAPN4, common to CAPN1 and CAPN2 [12]; deletion of the gene encoding CAPN4, *CAPNS4*, renders both CAPN1 and CAPN2 catalytically inactive [13]. While calpain cleavage is known as a highly regulated event, it does not occur at a rigidly defined site within a protein's amino acid sequence, but instead is dependent upon the primary sequence as well as secondary [14] and three-dimensional structure [15].

In this study, we describe cleavage of STIM1 both under endogenous conditions and in response to cell stress events. When calpain activity was inhibited, STIM1 cleavage was significantly reduced. Cleavage took place over a time course of hours to days, suggesting it does not occur at sufficient speed as to serve as a direct regulator of the process of SOCE. Instead, we propose that STIM1 is targeted for calpain cleavage as a mechanism to regulate the steady state levels of STIM1 within the cell. Furthermore, STIM1's susceptibility to calpain cleavage leads to enhanced STIM1 degradation during the progression of apoptosis.

2. Materials and methods

2.1. Protein synthesis, cell culture, SDS–PAGE, and immunoblotting

STIM1C protein was synthesized as described previously [16]: cDNA encoding the cytoplasmic domain of STIM1 (residues 271–

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* Corresponding author. Fax: +1 780 492 0886.
E-mail address: marek.michalak@ualberta.ca (M. Michalak).

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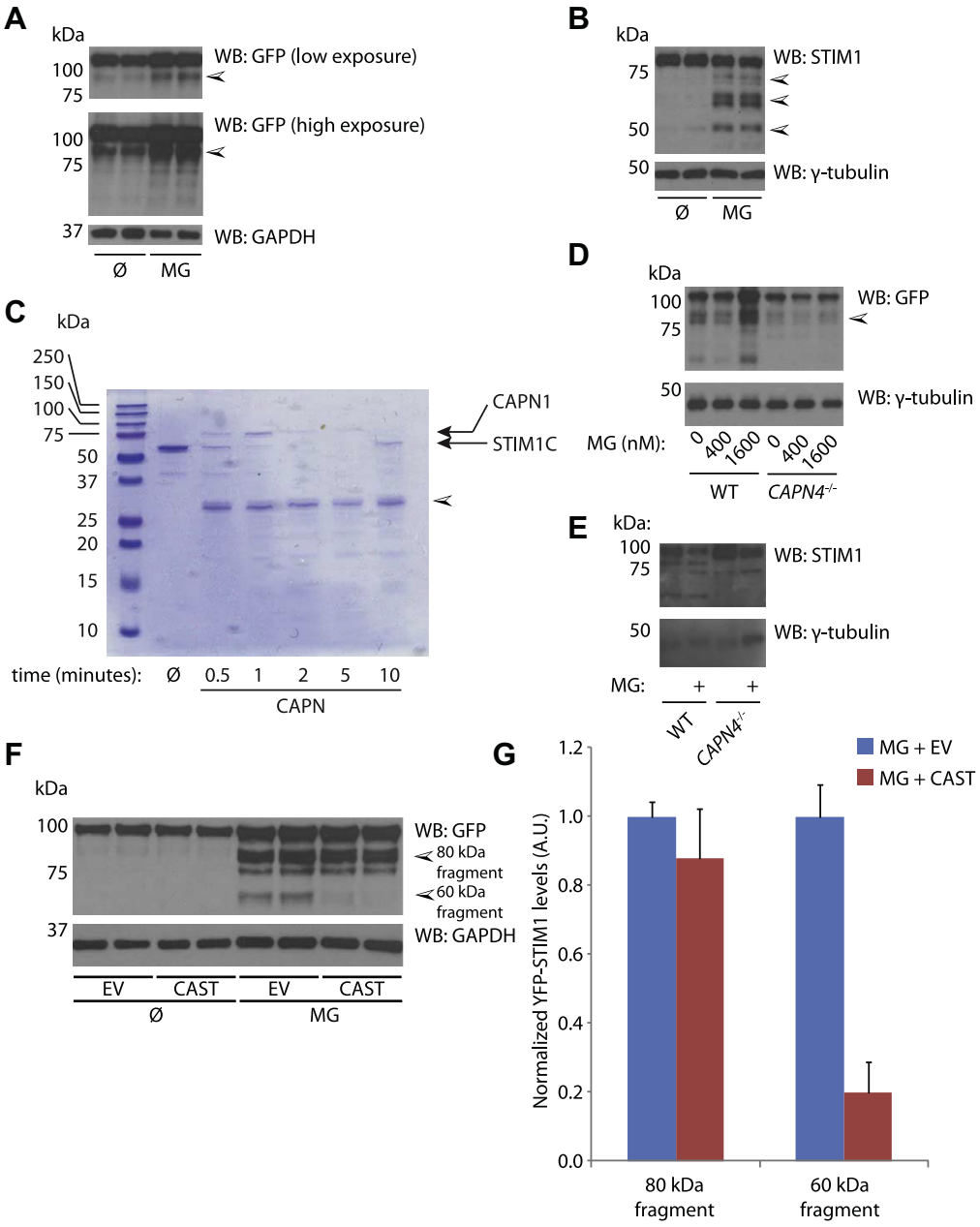

Fig. 1. STIM1 is cleaved by calpains. (A) *STIM1*$^{-/-}$ MEFs were transfected with a vector encoding YFP-STIM1 and left untreated or treated with 500 nM MG132 (MG). Lysates were separated via SDS–PAGE, and immunoblotted for GFP and GAPDH (loading control). MG132 treatment increased levels of lower molecular fragments of YFP-STIM1 (indicated by arrowheads). (B) Wild-type MEFs were left untreated or treated with 500 nM MG132 (MG). MG132 treatment increased abundance of lower molecular weight STIM1 fragments (indicated by arrowheads). γ-Tubulin served as a loading control. (C) STIM1C, purified from *E. coli*, was incubated with calpain protein in the presence of Ca^{2+} for time points up to 10 min as indicated in the Figure. Reactions were stopped by addition of sample loading buffer, after which samples were separated via SDS–PAGE and stained with Coomassie blue. Addition of CAPN1 led to cleavage of STIM1C almost immediately, as shown by diminished levels of full-length STIM1C and the presence of new bands with increased electrophoretic mobility as compared to the full-length STIM1C (indicated by arrowhead). (D) Wild-type and *CAPN4*$^{-/-}$ fibroblasts were transfected with a vector encoding YFP-STIM1 and left untreated, or treated with MG132 (400 nM or 1600 nM), as indicated. Lysates were separated via SDS–PAGE and probed with antibodies against GFP. Immunoblotting for GFP shows some cleavage of YFP-STIM1 under all conditions (indicated by arrowhead), with a marked increase at the higher MG132 concentration. Conversely, much less cleavage is seen in the absence of CAPN4, even when treated with 1600 nM MG132. γ-Tubulin served as a loading control. (E) Wild-type and *CAPN4*$^{-/-}$ fibroblasts were left untreated or treated with 500 nM MG132. MG132 increases production of lower molecular weight bands in wild-type cells but not in calpain-deficient cells. γ-Tubulin served as a loading control. (F) *STIM1*$^{-/-}$ MEFs were transfected with a vector encoding YFP-STIM1 and either empty vector or a vector encoding human calpastatin (CAST). Cells were then left untreated or treated with 500 nM MG132 (MG). Lysates were separated via SDS–PAGE and probed with an antibody against GFP. Co-expression of CAST inhibits production of lower molecular weight YFP-STIM1 fragments. Western blot against GAPDH served as a loading control. (G) Quantification of data in (F).

685) was generated via PCR-driven amplification using a STIM1-pCMV6-XL5 plasmid as template. The cDNA product was cloned into a pBAD/gIII *Escherichia coli* expression vector; recombinant STIM1C was expressed in *E. coli* and purified from lysates using Ni–NTA affinity column chromatography [17].

Generation and characterization of *STIM1*$^{-/-}$ cells were described earlier [16]. Cells were transfected with expression vectors encoding YFP-STIM1 and/or CAST using the Neon transfection system (Invitrogen). Protein concentration was estimated using a Lowry-based protein assay. SDS polyacrylamide gel electrophore-