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A novel cytoplasmic tail motif regulates mouse corin expression on the cell surface

Hui Li [a, 1], Yue Zhang [a, 1], Lina Wang [a], Ningzheng Dong [a, b], Xiaofei Qi [a, b, c], Qingyu Wu [a, d, *]

[a] Cyrus Tang Hematology Center, Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China
[b] Key Laboratory of Thrombosis and Hemostasis, Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, China
[c] Department of Urology, The First Affiliated Hospital of Soochow University, Suzhou, China
[d] Molecular Cardiology, Cleveland Clinic, Cleveland, OH, United States

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ABSTRACT

Type II transmembrane serine proteases (TTSPs) are important in many biological processes. Cell surface expression is critical for TTSP activation and function. To date, the mechanism underlying TTSP cell surface expression is poorly understood. Corin is a TTSP and acts as the pro-atrial natriuretic peptide convertase that is essential for sodium homeostasis and normal blood pressure. In this study, we investigated how cytoplasmic tail sequences may regulate corin expression and activation on the cell surface. By site-directed mutagenesis, we made mouse corin proteins with truncations or point-mutations in the cytoplasmic tail. We expressed the mutants in transfected HEK293 cells and analyzed corin cell surface expression and activation by Western blotting and flow cytometry. We found that corin truncation mutants lacking a Lys-Phe-Gln sequence at residues 71–73 had higher levels of cell surface expression and activation compared with that in wild-type corin. When Lys-71, Phe-72 and Gln-73 residues were mutated together, but not individually, in corin with the full-length cytoplasmic tail, increased levels of cell surface expression and zymogen activation were also observed. These results indicate that residues Lys-71, Phe-72 and Gln-73 serve as a novel retention motif in the intracellular pathway to regulate corin cell surface expression and activation.

1. Introduction

Type II transmembrane serine proteases (TTSPs) are trypsin-like enzymes that participate in diverse biological processes [1–5]. All TTSPs consist of an N-terminal cytoplasmic tail followed by a single-span transmembrane domain and an extracellular region with a C-terminal protease domain. The transmembrane domain serves as an anchor to restrict the protease activity in the desired tissues [1,6]. The cytoplasmic tails of the TTSPs range from 19 to 161 amino acids in length [1,6]. To date, their functional significance remains largely unknown.

Corin is a TTSP, consisting of a cytoplasmic tail, a transmembrane domain and an extracellular region with two frizzled, eight LDL receptor, a scavenger receptor and a protease domains [7,8]. In cardiomyocytes, corin activates the natriuretic peptides that are essential for normal blood volume and pressure [7,9–11]. Corin deficiency prevents natriuretic peptide processing and causes hypertension in knockout mice [12,13]. Corin variants have been found in people with hypertension [14–19].

Corin is synthesized in an inactive zymogen form. Upon reaching the cell surface, it is cleaved and activated at a conserved site [20–23]. Previously [24], we found that a DDNN cytoplasmic motif regulated human corin cell surface expression and zymogen activation, indicating that the cytoplasmic tail plays a role in corin intracellular trafficking.

The natriuretic peptides are conserved from fish species to mammals [25]. Corin is also conserved evolutionarily and its homolog exists in the fruit fly *Drosophila* [26,27]. Interestingly, the corin cytoplasmic tails in different species differ both in length and

Abbreviations: FBS, fetal bovine serum; HEK, human embryonic kidney; TTSP, type II transmembrane serine protease; WT, wild-type.

* Corresponding author. Lerner Research Institute, 9500 Euclid Avenue, Cleveland, OH 44195, United States.
E-mail address: wuq@ccf.org (Q. Wu).

[1] These authors contributed equally to this work.

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sequence (www.ensemble.org). For example, the mouse corin cytoplasmic tail is 67 amino acids longer than the human corin cytoplasmic tail [8]. Moreover, mouse and human corin cytoplasmic tails share little sequence similarities. In particular, mouse corin lacks the DDNN motif that mediates human corin cell surface expression [24].

We hypothesize that different cytoplasmic sequences may be involved in regulating mouse corin cell surface expression. To test this hypothesis, we created mouse corin proteins with altered cytoplasmic lengths and sequences. We expressed these proteins in human embryonic kidney 293 (HEK293) cells and analyzed their cell surface expression and zymogen activation by Western blotting and flow cytometry.

2. Materials and methods

2.1. Expression plasmids

Plasmids encoding mouse corin wild-type (WT) and mutants were constructed by site-directed mutagenesis. All plasmids were pcDNA3.1 (Invitrogen)-based and verified by sequencing. Individual corin mutants are illustrated in Figs. 1–4. Plasmids encoding human WT corin and a splicing variant, hE1a, were described previously [24]. All recombinant corin proteins contained a C-terminal V5 tag for protein detection in Western blotting and flow cytometry.

2.2. Cell culture and transfection

HEK293 cells were grown in 6-well plates (Corning) with DMEM and 10% fetal bovine serum (FBS) at 37 °C in humidified incubators with 5% CO_2 and 95% air. To express corin proteins, the cells were transfected with expression plasmids using TurboFect (Thermo Scientific) or FuGENE HD (Roche Diagnostics) reagents, following manufacturers' instructions. The cells were cultured in DMEM with 10% FBS at 37 °C for 24 h.

2.3. Western blotting

The transfected cells were lysed in a solution containing 50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100 (v/v), 10% glycerol (v/v), and a protease inhibitor mixture (1:100 dilution; Sigma).

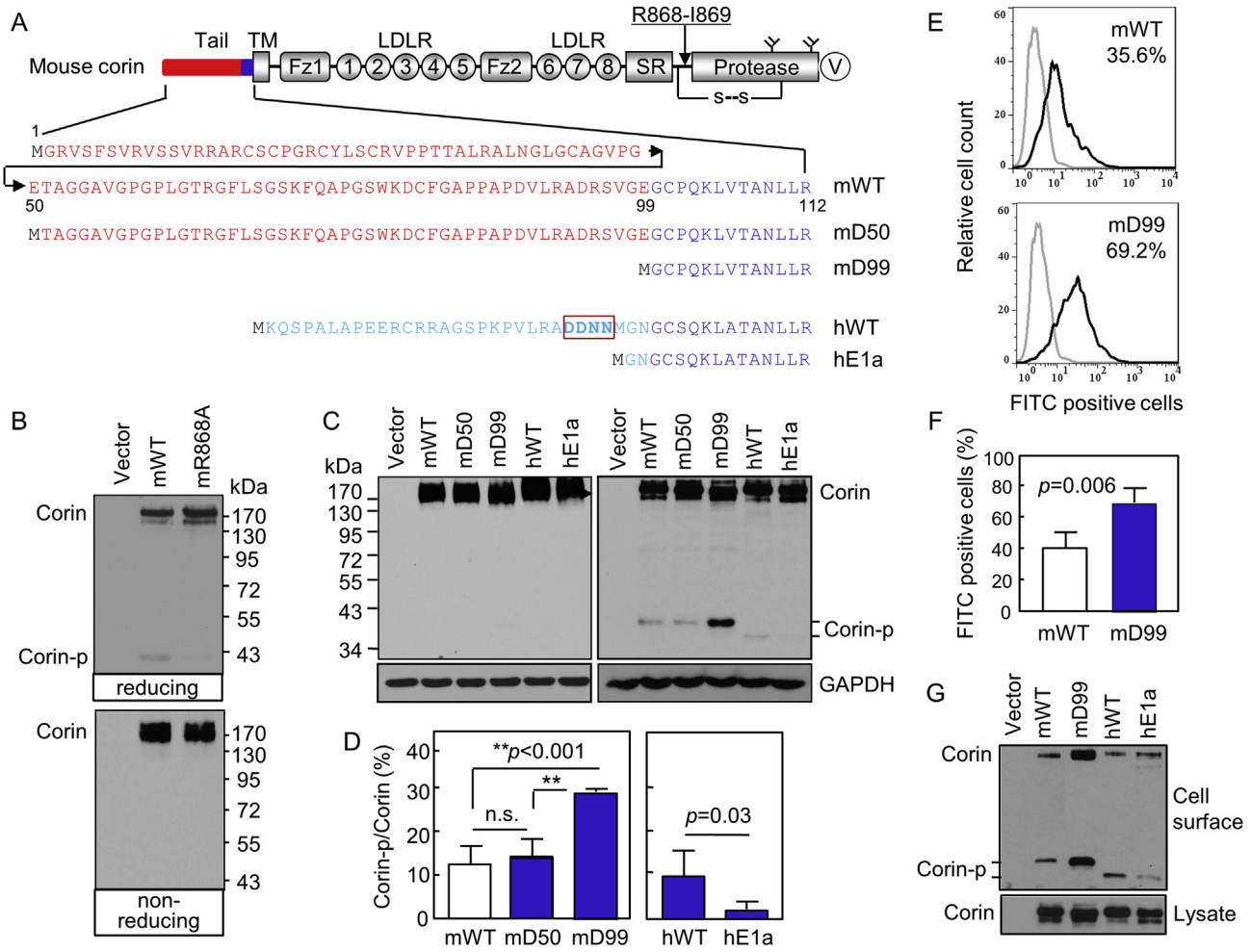

Fig. 1. Corin cytoplasmic tail and activation. (A) Corin domains and cytoplasmic sequences. An arrow indicates the activation site. A disulfide bond (s–s) links the protease domain and the propeptide. Y-shaped symbols in the protease domain indicate N-glycosylation sites. A V5 tag (V) is at the C-terminus. Cytoplasmic sequences in mouse (m) corin WT and the truncation mutants mD50 and mD99 as well as human (h) corin WT and the hE1a variant are shown. (B, C) Western analysis of corin proteins under reducing (B, top; C, right) and non-reducing (B, bottom; C, left) conditions. (D) Percentages of corin activation (Corin-p *vs.* Corin bands) were calculated based on densitometry of Western blots represented in C, right panel. n = 4 per group. $^{**}p < 0.001$; n.s., not significant. (E, F) Flow cytometric analysis of surface corin in HEK293 cells transfected with WT and mD99 plasmids. Data are mean ± S.D. of corin-positive cells. n = 4 per group. (G) Western analysis of cell surface-labeled corin proteins under reducing conditions (top). Corin proteins in cell lysates were controls (bottom).