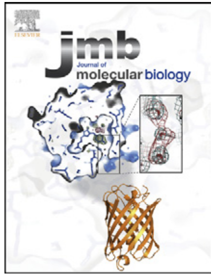

S100A10 Regulates ULK1 Localization to ER–Mitochondria Contact Sites in IFN-γ-Triggered Autophagy

Ying-Da Chen [1], Yi-Ting Fang [2], Chih-Peng Chang [1,2,3], Chiou-Feng Lin [3,4], Li-Jin Hsu [1,3,5], Shang-Rung Wu [6], Yen-Chi Chiu [6], Robert Anderson [7] and Yee-Shin Lin [1,2,3]

1 - ***Institute of Basic Medical Sciences,*** *College of Medicine, National Cheng Kung University, Tainan 701, Taiwan*
2 - ***Department of Microbiology and Immunology,*** *College of Medicine, National Cheng Kung University, Tainan 701, Taiwan*
3 - ***Center of Infectious Disease and Signaling Research,*** *College of Medicine, National Cheng Kung University, Tainan 701, Taiwan*
4 - ***Department of Microbiology and Immunology,*** *College of Medicine, Taipei Medical University, Taipei 110, Taiwan*
5 - ***Department of Medical Laboratory Science and Biotechnology,*** *College of Medicine, National Cheng Kung University, Tainan 701, Taiwan*
6 - ***Institute of Oral Medicine,*** *College of Medicine, National Cheng Kung University, Tainan 701, Taiwan*
7 - ***Departments of Microbiology & Immunology and Pediatrics,*** *and Canadian Center for Vaccinology, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada*

Correspondence to Yee-Shin Lin: *Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan. yslin1@mail.ncku.edu.tw*
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Abstract

During the process of autophagy, the autophagy-related proteins are translocated to autophagosome formation sites. Here, we demonstrate that S100A10 is required for ULK1 localization to autophagosome formation sites. Silencing of *S100A10* reduces IFN-γ-induced autophagosome formation. We also determined the role of annexin A2 (ANXA2), a binding partner of S100A10, which has been reported to promote phagophore assembly. Silencing of *ANXA2* reduced S100A10 expression. However, overexpression of S100A10 in *ANXA2*-silenced cells was still able to enhance autophagosome formation, suggesting that ANXA2 regulates IFN-γ-induced autophagy through S100A10. We also observed that S100A10 interacted with ULK1 after IFN-γ stimulation, and S100A10 knockdown prevented ULK1 localization to autophagosome formation sites. Finally, the release of high mobility group protein B1, one of the functions mediated by IFN-γ-induced autophagy, was inhibited in *S100A10* knockdown cells. These results elucidate the importance of S100A10 in autophagosome formation and reveal the relationship between S100A10 and ULK1 in IFN-γ-induced autophagy.

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Introduction

Autophagy is a ubiquitous process by which cells maintain biological functions by degrading their constituents. During the process of autophagy, cargos are engulfed by a membrane vesicle called an autophagosome, which is derived from a phagophore [1]. Autophagy-related (ATG) protein recruitment and vesicle trafficking are required for autophagosome formation [2]. Among the ATG proteins, the unc-51 like autophagy activating kinase 1 (ULK1) complex is commonly considered to be an initiator for autophagosome formation [3,4]. The activation of the ULK1 complex is inhibited and phosphorylated by the mechanistic target of the rapamycin (MTOR) complex. During autophagy induction, the MTOR complex is released from the ULK1 complex. Thus, ULK1 can trigger downstream signaling by phosphorylating ATG proteins such as ATG9 and Beclin 1 (BECN1) [5–8]. BECN1 is a component in the class III phosphatidylinositol 3-kinase (PI3K) complex, which phosphorylates phosphatidylinositol to phosphatidylinositol 3-phosphate and is required for protein recruitment in autophagosome formation [9]. During the elongation of the autophagosome, ATG5 forms a complex with ATG12 and ATG16. This

complex is required for LC3 conversion [2,10]. In mammalian cells, autophagosome formation occurs at the endoplasmic reticulum (ER)–mitochondria contact site within the mitochondria-associated ER membrane [11]. The sources of the autophagosome membrane in clude the ER, mitochondria, recycling endosomes, and plasma membrane [12–15]. However, the vesicular assembly of the autophagosome is still not fully resolved. Vesicle trafficking is important for autophagosome membrane extension. Furthermore, ATG protein translocation also relies on vesicle trafficking [16,17]. Since autophagosome formation depends on vesicle trafficking, it is likely that trafficking-related proteins are required for autophagy induction.

S100A10, also known as p11 or annexin A2 (ANXA2) light chain, is highly expressed in various kinds of cells and tissues such as lungs, intestines, and kidneys. It is a member of the S100 protein family and constitutively binds ANXA2 to form a heterotetramer [18,19]. The S100A10/ANXA2 complex forms symmetric junctions between opposing membrane surfaces to induce vesicle aggregation. When only one membrane surface is present, two ANXA2 subunits bind to the same membrane surface, while S100A10 can interact with other proteins [19]. The functions of S100A10 are linked with protein targets and vesicle trafficking. S100A10 regulates the function and expression of several receptors and channels such as the two-pore domain acid-sensitive K^+ channel-1 (TASK-1), transient receptor potential cation channel subfamily V member 5, transient receptor potential cation channel subfamily V member 6, and 5-hydroxytryptamine 1B [20–22]. Thus, S100A10 is important in various physiological processes. The S100A10/ANXA2 complex also regulates the endosomal recycling pathway and multivesicular endosome biogenesis [23,24]. Depletion of this complex affects the distribution and morphology of recycling endosomes. ANXA2 is secreted by an unconventional secretion pathway due to the lack of signal peptide. We previously showed that IFN-γ-induced surface expression of ANXA2 is associated with the exosomal secretion pathway and that the depletion of S100A10 inhibited exosomal secretion of ANXA2 [25].

Although autophagy is a well-known process to degrade intracellular constituents, it also plays an important role in unconventional secretion. Autophagy-mediated protein secretion involves the engulfment of protein targets by the autophagosome, which further fuses with lysosomes or multivesicular endosomes or directly with the plasma membrane. Following the fusion with the plasma membrane, protein targets are released extracellularly [26,27]. In mammalian cells, autophagy has been shown to induce high mobility group protein B1 (HMGB1) secretion [28]. HMGB1, a DNA-binding protein, can serve as an alarm to trigger the immune system [29]. Furthermore, it has been reported that IFN-γ can induce HMGB1 release [30]. Besides HMGB1 release, autophagy is also associated with various physiological processes and immune responses such as virus infection and cell differentiation and proliferation [31–35].

The functions of ATG proteins have been intensively studied. However, the translocation of ATG proteins in autophagosome formation is less clear. S100A10 has been known to be responsible for protein translocation and vesicle trafficking. Furthermore, S100A10 expression is induced by IFN-γ stimulation [25]. In this study, we investigated the role of S100A10 in IFN-γ-induced autophagy. Our data suggest that S100A10 is essential for the ULK1 localization to autophagosome formation sites after IFN-γ stimulation. Furthermore, depletion of S100A10 inhibited HMGB1 release, indicating that S100A10 causes autophagy-associated cell physiological effects.

Results

S100A10 is essential for IFN-γ-induced autophagy in human lung epithelial cells

S100A10 expression is induced by IFN-γ stimulation [25]. We examined the relationship between S100A10 and IFN-γ-induced autophagy in human lung epithelial Beas-2B cells. During the autophagy process, LC3-I is converted to LC3-II, which further accumulates in the autophagosomal membrane. Thus, LC3-II levels are increased in autophagy induction. Time-course analysis revealed that the levels of S100A10 and LC3-II were increased after cells were treated with 500 U/ml of IFN-γ (Supplementary Fig. 1a and b). Furthermore, we examined LC3 punctuation to monitor autophagosome formation, which showed an increase after IFN-γ treatment for 24 h (Supplementary Fig. 1c and d). We used tandem fluorescently tagged LC3 and detected increased autolysosome formation after IFN-γ treatment for 24 h (Supplementary Fig. 1e and f). LC3-II levels were further enhanced by IFN-γ treatment combined with autophagic flux inhibitors such as bafilomycin A1 and chloroquine (Supplementary Fig. 1g). In contrast, LC3-II levels were decreased by IFN-γ treatment combined with protein synthesis inhibitor such as cycloheximide (Supplementary Fig. 1h). Furthermore, upregulation of cathepsin S, a downstream signaling factor of IFN-γ, showed IFN-γ signaling induction (Supplementary Fig. 1i). These results indicate that IFN-γ elicited cellular autophagic activity in Beas-2B cells.

In the IFN-γ-treated *S100A10* knockdown cells, the number of LC3 puncta was decreased compared with control knockdown cells (Fig. 1a and b). The reduction of autophagosome formation was

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