論文 / 著書情報
Article / Book Information

題目(和文)	脱ユビキチン化酵素USP8およびSTAMBPL1の活性制御機構およびその生理的病理的意義の解明
Title(English)	Cis-regulations of USP8 and STAMBPL1 deubiquitinases and their pathophysiological roles
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種別(和文)	論文要旨
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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	柿原慧遵		

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指導教員（主）： Academic Supervisor(main)	藤田尚信
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Introduction

Protein ubiquitination is balanced via ubiquitination and deubiquitination. The latter are catalyzed by deubiquitinases (DUBs). Most DUBs are multi-domain proteins including catalytic domain. Dysregulations of DUB activity are associated with many human diseases[1]. In this study, I focused on two DUBs, ubiquitin-specific protease 8 (USP8) and STAM-binding protein-like 1 (STAMBPL1) in terms of their cis-regulation. USP8 plays roles in membrane trafficking pathways such as endocytosis. I previously identified some somatic mutations of USP8 gene in Cushing's disease (CD)[2]. These mutations are gain-of-function mutations, thus enhancing USP8 activity. STAMBPL1 is also known as an endosomal DUB. In addition, recent studies report that STAMBPL1 is highly expressed in multiple cancer types and it function as an oncogenetic protein[3]. While these DUBs are potentially related to the disease onsets, the molecular basis of their activity regulations remains unclear. Here, I share novel insights into these activity regulations and their pathophysiological roles in terms of the cis-regulation.

Results

Our previous study identified an intrinsic WW-like domain in USP8 as an autoinhibitory region. My in vitro pull-down assay showed that the WW-like domain interacts with its catalytic USP domain. Further in silico structural analysis indicated that the WW-like domain contacts and blocks the ubiquitin-binding pocket in the USP domain. 14-3-3 bind to USP8 in a phospho-dependent manner, then suppressing USP8 activity[4]. Incubation of FRET probe, which monitors the interaction between the WW-like and USP domains, with a 14-3-3 inhibitor resulted in a decrease of their interaction, indicating that 14-3-3 enhances the autoinhibition. A hotspot of CD-associated USP8 mutations locates at the 14-3-3-binding motif and these mutations cause 14-3-3 dissociations from USP8. My FRET analysis also showed that the representative CD-associated mutation (ΔS718) significantly decreased the autoinhibition. A recent paper reports a novel USP8 mutant (G664R) at the WW-like domain in CD patient[5]. Consistent with other CD-associated mutations, my data showed that G664R mutation dramatically increased USP8 activity. Using FRET probe, I also found that this mutation decreased the autoinhibition. Together with these results, I concluded that both CD-associated USP8 mutations relieve the autoinhibition, enhancing USP8 activity and causing CD.

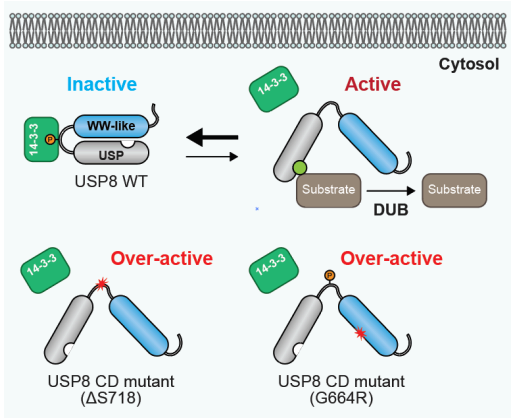

Fig.1 USP8 autoinhibition and CD

STAMBPL1 has a putative caspase cleavage site which is highly conserved in vertebrates. My immunoblotting data showed that STAMBPL1 is cleaved by caspases upon induction of apoptosis, then producing a cleaved C-terminal fragment consisting of only its catalytic JAMM domain, namely Ct-

STAMBPL1. My in silico structural analysis showed that STAMBPL1 N-terminal MIT domain functions as an autoinhibitory region by intramolecularly blocking a ubiquitin-binding pocket in the JAMM domain, suggesting that Ct-STAMBPL1 is relieved from the autoinhibition. Ct-STAMBPL1 exposes Gly residue, a target for myristoylation, on the N-terminus. My immunofluorescence data showed that Ct-STAMBPL1 clearly localizes on the plasma membrane while full-length on the cytosol and nucleus. My immunoblotting data also suggested that a part of Ct-STAMBPL1 has a high electromobility, a putative band of myristoylated Ct-STAMBPL1. Ct-STAMBPL1 overexpression in the cultured cells significantly induced apoptosis dependent on its catalytic activity. However, treatment with pan-caspase inhibitor cancelled Ct-STAMBPL1-dependent apoptosis, implying that Ct-STAMBPL1 induced apoptosis through caspases activation cascades even though STAMBPL1 cleavage is downstream of caspases activation. Taken together, I found that STAMBPL1 is cleaved by caspases during apoptosis and then exerts a pro-apoptotic activity on the plasma membrane.

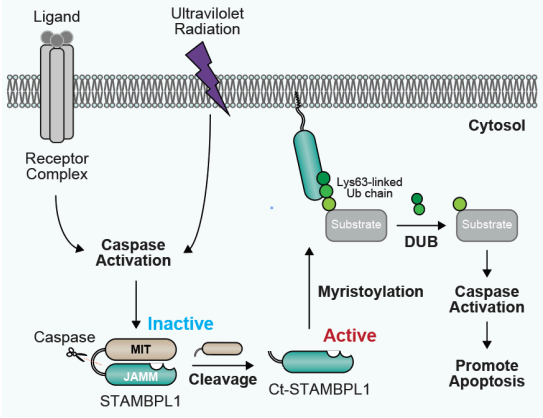

Fig.2 STAMBPL1 autoinhibition and apoptosis

Discussions

In general, many proteases full catalytic activity is tightly regulated just before they are required. It is well-characterized by the cis-regulation mechanism of some proteases. However, some attention has been only recently paid to these regulations of DUBs. Why do these DUBs have cis-regulation for tuning their activity? In terms of physiology, I speculate its importance on endosomal cargo sorting. Some researchers assume that USP8 and STAMBPL1 are activated on the endosome and involved in ubiquitinated cargo sorting. The cargo deubiquitination during endocytosis determines their destinations from the endosome; deubiquitination in the early stage for their recycling to the plasma membrane and in the late stage for their lysosomal degradation. According to this, the cis-regulations for USP8 and STAMBPL1 might be critical for timely deubiquitinating proper substrates on the endosome to sort them to their desired destinations. In actual, a constitutive-active USP8 mutant causes excessive cargo recycling to the plasma membrane. On the other hand, this study also focused on the relationship between the cis-regulation and the disease; dysfunction of USP8 autoinhibition and CD. Therefore, understanding DUB cis-regulations would no longer only provide us their physiological importance but also illuminate the pathogenesis of DUB-associated diseases and shed light on developing their therapy.