論 文 内 容 の 要 旨
Synopsis of Thesis

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論文題名 Title	Identification of CUL4A-DDB1-WDFY1 as an E3 ubiquitin ligase complex involved in initiation of lysophagy （リソファジー開始に関わるユビキチンE3リガーゼであるCUL4A-DDB1-WDFY1の同定）

論文内容の要旨

〔目 的(Purpose)〕

Macroautophagy is a bulk degradation system in which double membrane-bound structures called autophagosomes to deliver cytosolic materials to lysosomes. Autophagy promotes cellular homeostasis by selectively recognizing and sequestering specific targets, such as damaged organelles, protein aggregates, and invading bacteria, termed selective autophagy. We previously reported a type of selective autophagy, lysophagy/endophagy, which helps clear damaged lysosomes/endosomes. Damaged lysosomes/endosomes become ubiquitinated and recruit autophagic machinery. The SCFFBXO27 ubiquitin ligase complex, which is expressed mainly in the brain and muscle, ubiquitinates damaged lysosomal proteins by binding lumenal glycoproteins. However, a ubiquitously expressed E3 ligase involved in lysophagy/endophagy remains unclear. In addition, how cells recognize damaged membranes and the subsequent ubiquitination mechanisms have not been fully elucidated.

〔方法ならびに成績(Methods/Results)〕

To search for proteins playing an essential role in the initial damage recognition step of lysophagy, we performed proteomic analysis using different types of transfection reagent-coated polystyrene beads. When beads are coated with effectene, endocytosed beads induce endosomal membrane damage and are ubiquitinated. On the other hand, beads coated with polyethyleneimine (PEI) did not trigger severe endosomal membrane damage nor induce ubiquitination. Comparing these conditions, we detected proteins specifically recruited to damaged endosomes around beads, and 123 proteins with a high score were analyzed for Gene ontology enrichment (Table S1) and taken for further analysis.

To evaluate if any of the 123 proteins were involved in an early step of lysophagy, siRNA knockdown of these candidates was performed, and the GFP-Atg5 dot formation was monitored in response to lysosomal damage. Atg5 is one of autophagic machineries and is recruited to damaged lysosomes/endosomes in an early step of lysophagy/endophagy. As a result, CUL4A-DDB1-WDFY1 complex was identified as a candidate of E3 ubiquitin ligase involved in lysophagy/endophagy. Under each components knockdown conditions, the number of GFP-Atg5 dot decreased in response to lysosomal damage, suggesting that the recruitment mechanism of Atg5 did not work. In contrast, CUL4A-DDB1-WDFY1 complex was not involved in other types of autophagy such as starvation-induced autophagy and mitophagy (selective autophagy for damaged mitochondria).

Further research revealed that this complex is involved in the activity of endophagy/lysophagy (clearance of damaged lysosomes/endosomes), is recruited to damaged lysosomes/endosomes, and regulates K48-linked poly ubiquitination on damaged lysosomes/endosomes. We also revealed that WDFY1, which act as a 'receptor' in the CUL4A complex to recognize substrates to ubiquitinate and binds to luminal region of LAMP2 protein on damaged lysosomes, and this interaction is necessary for the recruitment of CUL4A-DDB1-WDFY1 complex to damaged lysosomes and the ubiquitination of LAMP2.

〔総 括(Conclusion)〕

In this study, we identified the CUL4A-DDB1-WDFY1 E3 ligase complex as a lysophagy regulator and found that this complex, upon lysosomal damage, ubiquitinated LAMP2. The CUL4A complex may contribute to the recruitment of autophagy receptor/adaptor proteins and autophagy-related proteins such as LC3 through LAMP2-ubiquitination. In intact cells, the CUL4A complex locates in the cytosol, which spatially separates from the lumen region of LAMP2. In our model, once the lysosomal membrane is injured, the lumenal side would be exposed to the cytosol and targeted for ubiquitination. This study contributes to the understanding of a mechanism of how cells tag only damaged lysosomes.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文は、損傷を受けたエンドソーム／リソソームを選択的に除去する選択的オートファジー（リソファジー）の開始段階に必要なユビキチン化酵素と、ユビキチン化酵素がダメージを受けたリソソームを認識するメカニズムを明らかにした研究成果をまとめたものである。

まずプロテオミクスにより、損傷を受けたエンドソーム／リソソームに集積するタンパク質が同定された。同定された因子に対するsiRNAを用いた、リソファジー活性に着目したスクリーニングにより、ユビキチンE3リガーゼであるCUL4A-DDB1-WDFY1複合体（CUL4A複合体）が見出された。さらなる検討により、リソソーム膜の損傷に応じてWDFY1がリソソーム膜タンパク質LAMP2の内腔側の構造を認識すること、CUL4A複合体がLAMP2にK48ユビキチン鎖を付加すること、K48ユビキチン鎖がオートファジーの初期因子の集積に必要であること、およびこのメカニズムがリソファジーの活性化に必要であることが明らかになった。

リソソームの恒常性の破綻は種々の疾患と関連することが知られており、本研究はリソソームの恒常性維持に必要なリソファジーのメカニズムの一端を明らかにした。よって、本論文は申請者 寺西宏文の学位論文に値すると判断された。