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Research Highlight

C-terminal amide-bearing proteins: emerging targets for protein homeostasis in health and disease

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ABSTRACT

This study highlights the role of C-terminal amide-bearing proteins (CTAPs) in protein homeostasis. Researchers discovered that CTAPs, formed under oxidative stress, are selectively recognized and degraded by the E3 ubiquitin ligase FBXO31 to maintain cellular health. Mutations in FBXO31, such as D334N, impair CTAP recognition, leading to abnormal protein accumulation and neurodevelopmental disorders. These findings not only elucidate the mechanism of protein quality control but also suggest potential therapeutic targets for diseases related to oxidative stress and protein misregulation.

In a recent study published in *Nature*, Matthias et al.[1] reported that C-terminal amide-bearing proteins (CTAPs) formed under oxidative stress of hydroxyl radicals are selectively recognized and degraded by FBXO31 to maintain cellular homeostasis. They also discovered that a human gene mutation associated with neurodevelopmental disorders impairs CTAPs recognition, highlighting the importance of FBXO31 in protein quality control.

When cells are exposed to external stress, such as oxidative damage, the degradation of damaged proteins is essential for maintaining normal cellular function. The ubiquitin-proteasome system is a major pathway for protein degradation, accounting for approximately 80% of intracellular protein turnover. E3 ubiquitin ligases are key components of this system, with over 600 human E3 ligases identified to date.[2] These enzymes recognize specific post-translational modifications on proteins, such as Lys48-linked polyubiquitination, to target them for degradation.[3] However, identifying chemically marked proteins that trigger selective protein degradation remains challenging.

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In this study, the researchers used a semi-synthetic fluorescent reporter system to evaluate the degradation of proteins with various chemical modifications, including tyrosine oxidation, carbonylation, advanced glycation end products, carbamylation, and C-terminal amides. They found that C-terminal amides, formed through protein cleavage under oxidative stress, are rapidly degraded via the ubiquitin-proteasome system. A CRISPR-Cas9 screen identified FBXO31 as the mediator of this process. FBXO31 is a substrate receptor for the SCF (SKP1–CUL1–F-box protein) ubiquitin ligase complex and specifically recognizes CTAPs for subsequent proteasomal degradation. FBXO31 has high affinity and selectivity for various C-terminal amides, but it does not possess this characteristic for unmodified C-termini and side-chain amides. Further investigation revealed that FBXO31 broadly interacts with CTAP-modified proteins rather than relying on specific protein sequences. Mechanistically, FBXO31 recognizes CTAPs through a conserved binding pocket that selectively binds the primary amide at the C-terminus while excluding unmodified carboxylic acids. In this process, the hydrophobic residues Y309 and I337, as well as the hydrogen-bonding residue T343 are crucial for its interaction with target proteins. This selective recognition is crucial for the degradation of oxidatively damaged proteins and the maintenance of cellular homeostasis. The study also explored the formation of CTAPs. While CTAPs are typically formed through enzymatic amidation in secretory peptides, the researchers discovered that intracellular CTAPs primarily arise from protein cleavage fragments induced by oxidative stress. Additionally, researchers found that FBXO31 has a strong response to oxidative stress, with associated proteins in the complex being upregulated under oxidative stress conditions. This finding suggests that FBXO31 serves as a quality control factor to eliminate protein fragments generated under oxidative stress conditions (Fig. 1A).

A pathogenic mutation in *FBXO31* has been linked to recessive intellectual disability. Two studies have also identified a *de novo* D334N mutation in *FBXO31* as a dominant genetic alteration among patients with diplegic spastic cerebral palsy.[4] This mutation causes FBXO31 to no longer recognize amidated proteins, leading to the accumulation of abnormal proteins. *In vitro* experiments showed that the D334N mutation eliminated FBXO31's activity toward CTAP substrates and instead led to strong ubiquitylation of the new substrate SUGT1. More importantly, this mutation alters the binding affinity of FBXO31, causing its substrate binding to shift from amidated proteins to proteins that bear the Lys/Arg-

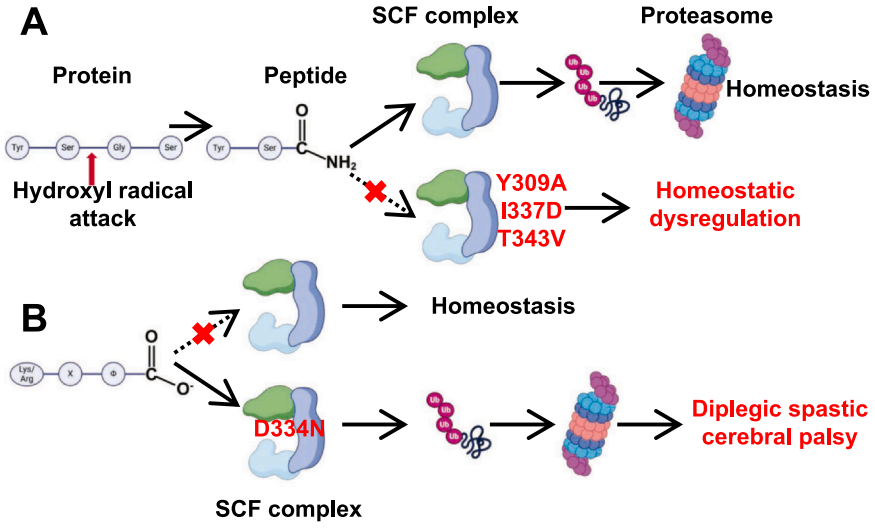

Fig. 1. FBXO31 maintains protein homeostasis by CTAPs in health and disease. (A) FBXO31 promotes the degradation of chemically modified proteins by recognizing C-terminal amides that form at the C-termini of peptide fragments generated by hydroxyl radicals. In this process, the hydrophobic residues Y309 and I337, as well as the hydrogen-bonding residue T343 are crucial for its interaction with target proteins. Mutations at these sites can lead to the abnormal accumulation of damaged proteins, thereby causing disease. (B) Normal proteins with carboxyl termini are not recognized by FBXO31. However, when FBXO31 undergoes a mutation at position 334 that eliminates the negative charge, it can bind to proteins with a Lys/Arg-X-Φ-COOH motif, leading to aberrant degradation. This mechanism has been identified in patients with cerebral palsy. Φ: hydrophobic residue; SCF: SKP1–CUL1–F-box protein complex; Ub: ubiquitin; X: any amino acid; Tyr: tyrosine; Ser: serine; Gly: glycine; Lys: lysine; Arg: arginine; Y: tyrosine; I: isoleucine; T: threonine; A: alanine; D: aspartic acid; V: valine; N: asparagine. (Created with BioRender.com).

X-Φ-COOH motif. This mutation redirects FBXO31 to degrade essential cellular proteins, such as SUGT1, a key regulator of mitotic spindle assembly. This indicates that the mutation at position 334 not only affects the activity of FBXO31, but also alters its substrate recognition pattern. Moreover, the mutation at position 334 significantly reduces the viability of cells. This misregulation of protein degradation likely contributes to neurodevelopmental disorders (Fig. 1B).

In summary, this study provides critical insights into how FBXO31 recognizes and degrades CTAPs to maintain cellular homeostasis. The findings also highlight the potential role of *FBXO31* mutations in neurodevelopmental disorders, opening new avenues for therapeutic interventions. Notably, in the context of cancer therapy, chemotherapy often induces oxidative stress in cells. In the mass spectrometry data from the authors regarding FBXO31 recognition of CTAPs, the well-known protein IDH1 in gliomas can be recognized and degraded by FBXO31 through its amidation.[5] This suggests that for patients with wild-type *IDH* status, the expression levels of IDH1 should also be monitored during chemotherapy, as it may potentially lead to changes in tumor subtypes. The discovery and elucidation of this oxidative stress-related protein homeostasis system may offer new insights into chemoresistance in cancer and potentially improve the efficacy of chemotherapy for cancer patients.

Future research should focus on further elucidating the molecular mechanisms of FBXO31-mediated CTAPs degradation and its roles in different cellular contexts, including aging, neurodegenerative diseases, and cancer. For example, inhibiting the degradation of CTAPs during chemotherapy might enhance the efficacy of chemotherapeutic agents. Meanwhile, targeting misfolded proteins in neurodegenerative diseases through this mechanism may provide new therapeutic strategies. Overall, leveraging the unique recognition of CTAPs by FBXO31 offers great potential for developing targeted protein degradation technologies. Future efforts should aim to enhance the specificity and efficiency of such technologies and explore their applications in drug development and personalized medicine.

CRediT authorship contribution statement

Chen Zhang: Writing – review & editing, Writing – original draft, Conceptualization. **Ruichen Zang:** Writing – original draft. **Yanlan Yu:** Writing – review & editing, Supervision. **Jie Zhang:** Writing – review & editing, Funding acquisition, Conceptualization, Supervision. **Guoqing Ding:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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