Report Title

Introduction

levantly reversible lipid modification (1). Our understanding of the enzymatic and biochemical aspects of S-acylation is largely due to the pioneering work of Thomas Graham. In Astex, we have successfully exploited large scale system biology methods to identify a biologically significant subset of the S-acylproteome, a challenge given the unique class of S-acylated proteins, which are relatively poorly annotated (2). Through a combination of experimental and in silico technologies, we have identified molecular pathways in which S-acylation is involved, revealing S-acylation as a novel yet shared mechanism in signalling networks and contexts as diverse as neurodegenerative diseases, tumorigenesis, and viral infection. Given the growing appreciation of the importance of these modifications in health, a deeper understanding of S-acylated proteins and their regulatory enzymes may uncover new targets for the treatment of these diseases.']

'Glycosylation is an important post-translational modification that modulates the stability, activity, and specific functions of proteins. Despite having been reported for decades, glycosylation's intricate connection to ubiquitination and degradation, despite having been well-studied on individual systems, has not been systematically surveyed across organisms. The majority of glycoproteins are degraded by the ubiquitin proteasome rather than the lysosome, so the connection may be substantial. This review surveys the connections between protein glycosylation, ubiquitination, and proteasomal degradation, highlighting the most pressing questions and experimental strategies to answer them.']

'Neuronal dendrites are the primary sites for excitatory synaptic input, and their structural and functional plasticity is thought to bestow the nervous system with the ability to learn and memorize. These sections elaborate on the molecular basis of protein degradation and its role in neuronal function, which has been systematically explored over the past decade and a half. Topics to be view in this section includes the role of the ubiquitin proteasome system, the calcium activated protease calpain, other proteases, and the regulation of the system under physiological and pathological conditions.']

'Overview of E2 Ub-conjugating enzymes and the ubiquitination pathway, focusing on the mechanistic features and regulatory principles, as well as the ways by which proteins are targeted for degradation and proteasome recognition. This will also include a critical review of the regulation of the E3 ligases, as well as the triage decision for substrates, and the implication of their alterations in diseases, concluding with the various basic and therapeutic interventions through drugs and small molecules in relation to this essential cellular degradation process.']

Literature

Literature Review:

Post-translational modifications (PTMs) play a crucial role in enriching the structural and functional diversity of cellular proteins, thereby impacting their stability and function within signaling pathways and disease development. The literature extensively explores the significance of PTMs, such as phosphorylation, acetylation, methylation, glycosylation, and ubiquitination, in regulating protein behavior.

Experimental evidence and in-depth analyses have highlighted the intricate impact of specific PTMs on protein stability and function. For instance, the literature discusses how methylation of specific lysine residues can either promote or suppress the activity of key proteins like p53, illustrating the complexity of PTMs in modulating protein behavior. Additionally, PTMs such as phosphorylation have been shown to intricately influence protein stability, exemplified by their role in regulating proteins like MYC and HIF-1α.

Moreover, the literature emphasizes the regulatory role of phosphorylation in ubiquitination and its influence on transcriptional activation functions, particularly within cellular signaling pathways. The crosstalk between different PTMs and their cumulative impact on protein stability and cellular signaling has been a focal point of analysis, shedding light on the intricate regulatory mechanisms governed by PTMs in cellular processes.

Furthermore, the review provides insights into the interplay of PTMs in regulating protein stability and function, underscoring their implications in the development of various diseases. The intricate mechanisms and crosstalk between PTMs and signaling cascades presented in the literature pave the way for a deeper understanding of their therapeutic potential in disease intervention.

The above literature collectively provides a comprehensive overview of the diverse landscape of PTMs and their profound influence on protein stability, function, and their implications in cellular signaling pathways and disease development.

References:

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10533364/pdf/
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10567573/pdf/
3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10545776/pdf/
4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10196180/pdf/
5. Additional sources not directly cited in the literature review:
- "With a limited number of genes, cells achieve remarkable diversity..."
- "Specificity within the ubiquitin-proteasome system is primarily achieved through E3 ubiquitin ligases..."
- "Regulated in development and DNA damage-response 1 (REDD1) is a stress-induced protein that controls various cellular functions..."

- "Bladder cancer is one of the common malignant urothelial tumors..."

Discussion

Based on the provided content and related papers, the discussion section of the paper focuses on the impact of post-translational modifications (PTMs) on the stability and function of proteins involved in cellular signaling pathways, and their role in the development of various diseases. The strengths and weaknesses of the approach to the topic are discussed with a critical analysis of the results of the research topic.

Strengths:

1. Thorough Analysis: The discussion provides a comprehensive analysis of various PTMs, including ubiquitination, SUMOylation, glycosylation, and methylation, and their impact on protein stability and function.
2. Integration of Findings: The discussion integrates findings from various studies to highlight the complexity of PTMs and their effects on protein stability and activity.
3. Future Research Directions: The discussion identifies unanswered questions and research gaps related to the physiological relevance of PTMs, providing a basis for future research directions in the field of protein stability and PTMs.

Weaknesses:

1. Lack of Clear Molecular Mechanisms: Despite considerable efforts, the articles acknowledge the challenge in elucidating clear molecular mechanisms underlying the impact of PTMs on protein stability, suggesting a limitation in the current understanding of this complex process.
2. Conflicting Evidence: The discussion highlights conflicting evidence regarding the effects of specific PTMs on protein stability and activity, suggesting a lack of consensus in the field.

Comparison with Other Studies:

The articles compare their findings with other studies in related fields, emphasizing the complexity and variability of PTMs and their effects on protein stability and function. They acknowledge the need for further research to reconcile conflicting evidence and establish a clearer understanding of the role of PTMs in cellular signaling pathways and disease development.

Future Research Directions:

Based on the findings and limitations of the current studies, future research directions are proposed, such as the exploration of additional PTMs that may control protein stability and the elucidation of the physiological relevance of PTMs. They also suggest the application of protein stability proteomics for studying PTMs and their impact on protein degradation pathways.

In summary, the discussion sections provide a detailed, comprehensive analysis of the impact of PTMs on protein stability and function, emphasizing the complexity and diversity of PTMs and their implications for cellular signaling pathways and disease development. They highlight the need for

further research to elucidate the physiological relevance of PTMs and their potential as therapeutic targets in the treatment of diseases.

References:

1. [Link to the first article]
2. [Link to the second article]
3. [Link to the third article]
4. [Link to the fourth article]
5. [Link to the fifth article]

Idea

Given the information provided, a potential research problem and rationale could be formulated as follows:

Problem: Investigating the role of specific post-translational modifications (PTMs) in the regulation of protein stability and their potential as novel drug targets for diseases lacking effective treatments.

Rationale: The existing literature highlights the essential role of PTMs in controlling protein stability through mechanisms such as PTM-activated and PTM-inactivated degrons. Additionally, insights from related studies demonstrate the significance of PTMs, such as S-acylation and ubiquitination, in cellular pathways and disease processes, including cancer development and therapeutic implications. Therefore, further investigation into the specific PTMs involved in regulating protein stability and their potential as druggable targets is crucial for advancing our understanding of disease mechanisms and identifying novel therapeutic strategies.

Method

Method:
To investigate the role of specific post-translational modifications (PTMs) in the regulation of protein stability and their potential as novel drug targets for diseases lacking effective treatments, the following comprehensive and innovative method is proposed:

Rationale:

The proposed method is structured with the objective of elucidating the specific PTMs influencing protein stability and their potential as druggable targets, aligning with the current gaps in understanding disease mechanisms and identifying novel therapeutic strategies. Based on the existing literature, the emphasis is on the essential role of PTMs, such as S-acylation and ubiquitination, in controlling protein stability and their implications in disease processes, including cancer development and therapeutic implications.

1. Literature Review:

- Conduct a systematic review of existing studies focusing on PTMs associated with protein stability, with a particular emphasis on PTM-activated and PTM-inactivated degrons. Additionally, explore the related studies to understand the diverse roles of PTMs, such as S-acylation and ubiquitination, in cellular pathways and disease processes, as demonstrated in the provided related paper titles and abstracts.

2. PTM Analysis:

- Utilize advanced mass spectrometry-based proteomics approaches to comprehensively identify and quantify PTMs of interest, particularly focusing on the specific amino acids within regulatory domains of target proteins. This analysis will aid in understanding the abundance, dynamics, and interplay of PTMs in regulating protein stability.

3. In vitro and In vivo Functional Studies:

- Employ cell culture models and genetically modified animal models to investigate the functional consequences of specific PTMs on protein stability. This may involve CRISPR/Cas9 technology to manipulate the PTM machinery for gaining insights into the regulatory roles of identified PTMs in protein stability under physiological and pathological conditions.

4. Structural Biology Studies:

- Use high-resolution structural biology techniques, including X-ray crystallography and cryo-electron microscopy, to elucidate the mechanistic details of how specific PTMs influence the conformation and stability of target proteins. This will provide critical insights into the molecular basis of PTM-mediated regulation of protein stability.

5. Drug Target Validation:

- Leverage computational and experimental screening approaches to identify small molecule modulators capable of targeting the specific PTMs identified as crucial regulators of protein stability. Validate the druggability of these targets through in vitro and cell-based assays to ascertain their potential as novel therapeutic interventions for diseases lacking effective treatments.

6. Therapeutic Implications:

- Integrate the findings from the above methods to delineate the therapeutic implications of targeting specific PTMs in diseases, particularly emphasizing those where effective treatments are currently lacking. This will involve assessing the potential of developing PTM-modulating therapeutics and their feasibility in preclinical and clinical settings.

This method is envisioned to provide a holistic understanding of the role of specific PTMs in regulating protein stability and to pave the way for the development of innovative therapeutic strategies targeting PTMs for diseases with unmet medical needs.

Experiment

of REDD1 are dictated by its cellular context and other interacting partners, leading to either homeostatic or pathophysiological outcomes. This review summarizes the current understanding of REDD1's pathological roles in various diseases and the mechanisms underlying its regulation of cellular functions. Potential therapeutic strategies involving the targeting of REDD1 in treating metabolic disorders, neurodegeneration, and cancer are also discussed.', 'Bladder cancer is one of the most common malignancies worldwide, with a high recurrence rate and poor prognosis. Dysregulation of the ubiquitin-proteasome system, particularly E3 ubiquitin ligases and deubiquitinases, plays a critical role in bladder cancer development and progression. E3 ligases promote the ubiquitination of substrate proteins for proteasomal degradation, whereas deubiquitinases counterbalance this process by removing ubiquitin molecules from substrates and stabilizing proteins. Various E3 ligases and deubiquitinases are dysregulated in bladder cancer, leading to aberrant ubiquitination of tumor suppressors and oncogenes. Targeting these dysregulated enzymes may represent a promising therapeutic strategy for bladder cancer. This review focuses on the role of E3 ubiquitin ligases and deubiquitinases in bladder cancer development and discusses the potential of targeting these enzymes for immunotherapeutic interventions.']

Entities:

1. Post-translational modifications
2. Protein stability
3. Degrons
4. Mass spectrometry-based proteomics
5. CRISPR/Cas9 technology
6. Structural biology techniques
7. Small molecule modulators
8. Ubiquitination
9. S-acylation
10. Cancer development
11. Diseases lacking effective treatments

Design of the Experiment based on the provided information:

Title: Investigating the Role of Specific Post-Translational Modifications in Regulating Protein Stability for Therapeutic Targeting

Objective:

To elucidate the impact of specific post-translational modifications (PTMs) on protein stability and evaluate their potential as novel drug targets for diseases lacking effective treatments.

Experimental Design:

1. Literature Review:

- Conduct a systematic review of existing studies focusing on PTMs associated with protein stability, particularly PTM-activated and PTM-inactivated degrons.
- Explore related papers on S-acylation, ubiquitination, and their relevance to cancer development and therapeutic implications.

2. PTM Analysis:

- Utilize mass spectrometry-based proteomics to identify and quantify specific PTMs involved in regulating protein stability, focusing on target proteins' regulatory domains.

3. In vitro and In vivo Functional Studies:

- Use cell culture and genetically modified animal models to investigate the functional role of identified PTMs in protein stability.
- Utilize CRISPR/Cas9 technology to manipulate PTM machinery and study the regulatory roles of PTMs under physiological and pathological conditions.

4. Structural Biology Studies:

- Employ X-ray crystallography and cryo-electron microscopy to elucidate the mechanisms by which specific PTMs influence protein conformation and stability.

5. Drug Target Validation:

- Implement computational and experimental screening to identify small molecule modulators targeting specific PTMs regulating protein stability.
- Validate the druggability of these targets through in vitro and cell-based assays.

6. Therapeutic Implications:

- Integrate findings from the above methods to assess the therapeutic potential of targeting specific PTMs in diseases lacking effective treatments.
- Evaluate the feasibility of developing PTM-modulating therapeutics for preclinical and clinical applications.

By following this experimental design, we aim to deepen our understanding of the role of specific PTMs in regulating protein stability and potentially identify novel therapeutic strategies for diseases with unmet medical needs.

More related paper

Paper 1
Title: Refining S-acylation: Structure, regulation, dynamics, and therapeutic implications.

Abstract: With a limited number of genes, cells achieve remarkable diversity. This is to a large extent achieved by chemical posttranslational modifications of proteins. Amongst these are the lipid modifications that have the unique ability to confer hydrophobicity. The last decade has revealed that lipid modifications of proteins are extremely frequent and affect a great variety of cellular pathways and physiological processes. This is particularly true for S-acylation, the only reversible lipid modification. The enzymes involved in S-acylation and deacylation are only starting to be understood, and the list of proteins that undergo this modification is ever-increasing. We will describe the state of knowledge on the enzymes that regulate S-acylation, from their structure to their regulation, how S-acylation influences target proteins, and finally will offer a perspective on how alterations in the balance between S-acylation and deacylation may contribute to disease.

DOI: 10.1083/jcb.202307103

The impact factor: 8.077

Paper 2

Title: Defining E3 ligase-substrate relationships through multiplex CRISPR screening.

Abstract: Specificity within the ubiquitin-proteasome system is primarily achieved through E3 ubiquitin ligases, but for many E3s their substrates-and in particular the molecular features (degrons) that they recognize-remain largely unknown. Current approaches for assigning E3s to their cognate substrates are tedious and low throughput. Here we developed a multiplex CRISPR screening platform to assign E3 ligases to their cognate substrates at scale. A proof-of-principle multiplex screen successfully performed ~100 CRISPR screens in a single experiment, refining known C-degron pathways and identifying an additional pathway through which Cul2(FEM1B) targets C-terminal proline. Further, by identifying substrates for Cul1(FBXO38), Cul2(APPBP2), Cul3(GAN), Cul3(KLHL8), Cul3(KLHL9/13) and Cul3(KLHL15), we demonstrate that the approach is compatible with pools of full-length protein substrates of varying stabilities and, when combined with site-saturation mutagenesis, can assign E3 ligases to their cognate degron motifs. Thus, multiplex CRISPR screening will accelerate our understanding of how specificity is achieved within the ubiquitin-proteasome system.

DOI: 10.1038/s41556-023-01229-2

The impact factor: 28.213

Paper 3

Title: The stress-responsive protein REDD1 and its pathophysiological functions.

Abstract: Regulated in development and DNA damage-response 1 (REDD1) is a stress-induced protein that controls various cellular functions, including metabolism, oxidative stress, autophagy, and cell fate, and contributes to the pathogenesis of metabolic and inflammatory disorders, neurodegeneration, and cancer. REDD1 usually exerts deleterious effects, including tumorigenesis, metabolic inflammation, neurodegeneration, and muscle dystrophy; however, it also exhibits protective functions by regulating multiple intrinsic cell activities through either an mTORC1-dependent or -independent mechanism. REDD1 typically regulates mTORC1 signaling, NF-ÎºB activation, and cellular pro-oxidant or antioxidant activity by interacting with 14-3-3 proteins, IÎºBÎ±, and thioredoxin-interacting protein or 75â■■kDa glucose-regulated protein, respectively. The diverse functions of REDD1 depend on cell type, cellular context, interaction partners, and cellular localization (e.g., mitochondria, endomembrane, or cytosol). Therefore, comprehensively understanding the molecular mechanisms and biological roles of REDD1 under pathophysiological conditions is of utmost importance. In this review, based on the published literature, we highlight and discuss the molecular mechanisms underlying the REDD1 expression and its actions, biological functions, and pathophysiological roles.

DOI: 10.1038/s12276-023-01056-3

The impact factor: 12.153

Paper 4

Title: The role of E3 ubiquitin ligases and deubiquitinases in bladder cancer development and immunotherapy.

Abstract: Bladder cancer is one of the common malignant urothelial tumors. Post-translational modification (PTMs), including ubiquitination, acetylation, methylation, and phosphorylation, have been revealed to participate in bladder cancer initiation and progression. Ubiquitination is the common PTM, which is conducted by E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin-protein ligase. E3 ubiquitin ligases play a key role in bladder oncogenesis and progression and drug resistance in bladder cancer. Therefore, in this review, we summarize current knowledge regarding the functions of E3 ubiquitin ligases in bladder cancer development. Moreover, we provide the evidence of E3 ubiquitin ligases in regulation of immunotherapy in bladder cancer. Furthermore, we mention the multiple compounds that target E3 ubiquitin ligases to improve the therapy efficacy of bladder cancer. We hope our review can stimulate researchers and clinicians to investigate whether and how targeting E3 ubiquitin ligases acts a novel strategy for bladder cancer therapy.

DOI: 10.3389/fimmu.2023.1202633

The impact factor: 8.786

Paper 5

Title: Precise Orchestration of Gasdermins' Pore-Forming Function by Posttranslational Modifications in Health and Disease.

Abstract: Gasdermins (GSDMs) serve as pivotal executors of pyroptosis and play crucial roles in host defence, cytokine secretion, innate immunity, and cancer. However, excessive or inappropriate GSDMs activation is invariably accompanied by exaggerated inflammation and results in tissue damage. In contrast, deficient or impaired activation of GSDMs often fails to promptly eliminate pathogens, leading to the increasing severity of infections. The activity of GSDMs requires meticulous regulation. The dynamic modulation of GSDMs involves many aspects, including autoinhibitory structures, proteolytic cleavage, lipid binding and membrane translocation (oligomerization and pre-pore formation), oligomerization (pore formation) and pore removal for membrane repair. As the most comprehensive and efficient regulatory pathway, posttranslational modifications (PTMs) are widely implicated in the regulation of these aspects. In this comprehensive review, we delve into the complex mechanisms through which a variety of proteases cleave GSDMs to enhance or hinder their function. Moreover, we summarize the intricate regulatory mechanisms of PTMs that govern GSDMs-induced pyroptosis.

DOI: 10.7150/ijbs.86869

The impact factor: 10.75

Paper 6

Title: Applications of genetic code expansion technology in eukaryotes.

Abstract: Unnatural amino acids (UAAs) have gained significant attention in protein engineering and drug development owing to their ability to introduce new chemical functionalities to proteins. In eukaryotes, genetic code expansion (GCE) enables the incorporation of UAAs and facilitates posttranscriptional modification (PTM), which is not feasible in prokaryotic systems. GCE is also a powerful tool for cell or animal imaging, the monitoring of protein interactions in target cells, drug development, and switch regulation. Therefore, there is keen interest in utilizing GCE in eukaryotic systems. This review provides an overview of the application of GCE in eukaryotic systems and discusses current challenges that need to be addressed.

DOI: 10.1093/procel/pwad051

The impact factor: 15.328

Paper 7

Title: Protein modification by short-chain fatty acid metabolites in sepsis: a comprehensive review.

Abstract: Sepsis is a major life-threatening syndrome of organ dysfunction caused by a dysregulated host response due to infection. Dysregulated immunometabolism is fundamental to the onset of sepsis. Particularly, short-chain fatty acids (SCFAs) are gut microbes derived metabolites serving to drive the communication between gut microbes and the immune system, thereby exerting a profound influence on the pathophysiology of sepsis. Protein post-translational modifications (PTMs) have emerged as key players in shaping protein function, offering novel insights into the intricate connections between metabolism and phenotype regulation that characterize sepsis. Accumulating evidence from recent

studies suggests that SCFAs can mediate various PTM-dependent mechanisms, modulating protein activity and influencing cellular signaling events in sepsis. This comprehensive review discusses the roles of SCFAs metabolism in sepsis associated inflammatory and immunosuppressive disorders while highlights recent advancements in SCFAs-mediated lysine acylation modifications, such as substrate supplement and enzyme regulation, which may provide new pharmacological targets for the treatment of sepsis.

DOI: 10.3389/fimmu.2023.1171834

The impact factor: 8.786

Paper 8

Title: Protein neddylation and its role in health and diseases.

Abstract: NEDD8 (Neural precursor cell expressed developmentally downregulated protein 8) is an ubiquitin-like protein that is covalently attached to a lysine residue of a protein substrate through a process known as neddylation, catalyzed by the enzyme cascade, namely NEDD8 activating enzyme (E1), NEDD8 conjugating enzyme (E2), and NEDD8 ligase (E3). The substrates of neddylation are categorized into cullins and non-cullin proteins. Neddylation of cullins activates CRLs (cullin RING ligases), the largest family of E3 ligases, whereas neddylation of non-cullin substrates alters their stability and activity, as well as subcellular localization. Significantly, the neddylation pathway and/or many neddylation substrates are abnormally activated or over-expressed in various human diseases, such as metabolic disorders, liver dysfunction, neurodegenerative disorders, and cancers, among others. Thus, targeting neddylation becomes an attractive strategy for the treatment of these diseases. In this review, we first provide a general introduction on the neddylation cascade, its biochemical process and regulation, and the crystal structures of neddylation enzymes in complex with cullin substrates; then discuss how neddylation governs various key biological processes via the modification of cullins and non-cullin substrates. We further review the literature data on dysregulated neddylation in several human diseases, particularly cancer, followed by an outline of current efforts in the discovery of small molecule inhibitors of neddylation as a promising therapeutic approach. Finally, few perspectives were proposed for extensive future investigations.

DOI: 10.1038/s41392-024-01800-9

The impact factor: 38.104

Paper 9

Title: Research on the biological mechanism and potential application of CEMIP.

Abstract: Cell migration-inducing protein (CEMIP), also known as KIAA1199 and hyaluronan-binding protein involved in hyaluronan depolymerization, is a new member of the hyaluronidase family that degrades hyaluronic acid (HA) and remodels the extracellular matrix. In recent years, some studies have reported that CEMIP can promote the proliferation, invasion, and adhesion of various tumor cells

and can play an important role in bacterial infection and arthritis. This review focuses on the pathological mechanism of CEMIP in a variety of diseases and expounds the function of CEMIP from the aspects of inhibiting cell apoptosis, promoting HA degradation, inducing inflammatory responses and related phosphorylation, adjusting cellular microenvironment, and regulating tissue fibrosis. The diagnosis and treatment strategies targeting CEMIP are also summarized. The various functions of CEMIP show its great potential application value.

DOI: 10.3389/fimmu.2023.1222425

The impact factor: 8.786

Paper 10

Title: Protein Stability Regulation in Osteosarcoma: The Ubiquitin-like Modifications and Glycosylation as Mediators of Tumor Growth and as Targets for Therapy.

Abstract: The identification of new therapeutic targets and the development of innovative therapeutic approaches are the most important challenges for osteosarcoma treatment. In fact, despite being relatively rare, recurrence and metastatic potential, particularly to the lungs, make osteosarcoma a deadly form of cancer. In fact, although current treatments, including surgery and chemotherapy, have improved survival rates, the disease's recurrence and metastasis are still unresolved complications. Insights for analyzing the still unclear molecular mechanisms of osteosarcoma development, and for finding new therapeutic targets, may arise from the study of post-translational protein modifications. Indeed, they can influence and alter protein structure, stability and function, and cellular interactions. Among all the post-translational modifications, ubiquitin-like modifications (ubiquitination, deubiquitination, SUMOylation, and NEDDylation), as well as glycosylation, are the most important for regulating protein stability, which is frequently altered in cancers including osteosarcoma. This review summarizes the relevance of ubiquitin-like modifications and glycosylation in osteosarcoma progression, providing an overview of protein stability regulation, as well as highlighting the molecular mediators of these processes in the context of osteosarcoma and their possible targeting for much-needed novel therapy.

DOI: 10.3390/cells13060537

The impact factor: 7.666