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ORIGINAL RESEARCH

Proteome-Wide Mapping of Artesunate Targets Reveals Enrichment of the Ubiquitin-Proteasome System

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Background: Artesunate (AS) has great pharmacokinetic and clinical value. However, a comprehensive and up-to-date study exclusively focusing on AS direct binding proteins has not yet been conducted.

Methods: We performed a systematic, data-driven mapping of AS-binding protein via HuProt™ 20K human proteome microarray. To characterize the biological features of AS-binding proteins through a series of bioinformatic analyses.

Results: Firstly, AS targeted ubiquitin-mediated proteolysis, mineral absorption, Salmonella infection and glycolysis/gluconeogenesis. Among them, ubiquitin-mediated proteolysis has highest confidence scores, chaperone complex and ubiquitin-like protein conjugating enzyme activity were enriched in this set. Secondly, we showed that the bioactivity of AS encompasses a multifaceted range of health-promoting effects. Collectively, this study provided a valuable resource for AS-binding proteins. Furthermore, protein biological function is determined by their three-dimensional structure, when a protein fails to fold into its native structure, the proteins undergo mislocalisation/abnormal accumulation/degradation, leading to conformational diseases (CDs).

Conclusion: Considering that AS could target ubiquitin-proteasome system (UPS) and encompass a multifaceted range of health-promoting effects, a comprehensive understanding of the regulatory effects of AS on the UPS and its intrinsic mechanisms will enhance its ability to serve as a protective agent to fight against CDs.

Keywords: artesunate, HuProt™ 20K human proteome microarray, ubiquitin-mediated proteolysis, bioactivities, conformation diseases

Introduction

The clinical success of artemisinin in treating malaria has prompted the development of numerous semi-synthetic analogues.[1] Among them, artesunate (AS) is the most extensively studied due to its enhanced water solubility and oral bioavailability.[2] AS demonstrated broad health-promoting effects across multiple systems, including the digestive, respiratory, circulatory, nervous, endocrine, urinary, and motor systems.[3]

Proteins rely on their precise three-dimensional structures to function. Failure to achieve this native conformation leads to misfolded proteins, ultimately causing conformational diseases (CDs).[4] To maintain homeostasis, cells employ chaperones and proteases, two autoregulatory systems. Notably, the ubiquitin-proteasome system (UPS), a specialized protease system, is responsible for degrading up to 90% of intracellular proteins.[5] While the bioactivities of AS are known, its direct protein targets and mechanistic links to CDs remain underexplored.

Growing evidence suggested that AS may exert its effects through the UPS. For instance, AS resistance in malaria has been linked to the parasite's unfolded protein response (UPR), a stress-regulatory pathway mediated by the UPS in *P. falciparum*.[6] In cancer contexts, the combination of AS and WNT974 was shown to promote KRAS degradation via upregulation of E3 ligases, such as ANACP2 and β-TrCP within the ubiquitin-proteasome pathway.[7] Furthermore, AS has been found to mitigate age-related intestinal barrier dysfunction by reducing endoplasmic reticulum stress and UPR

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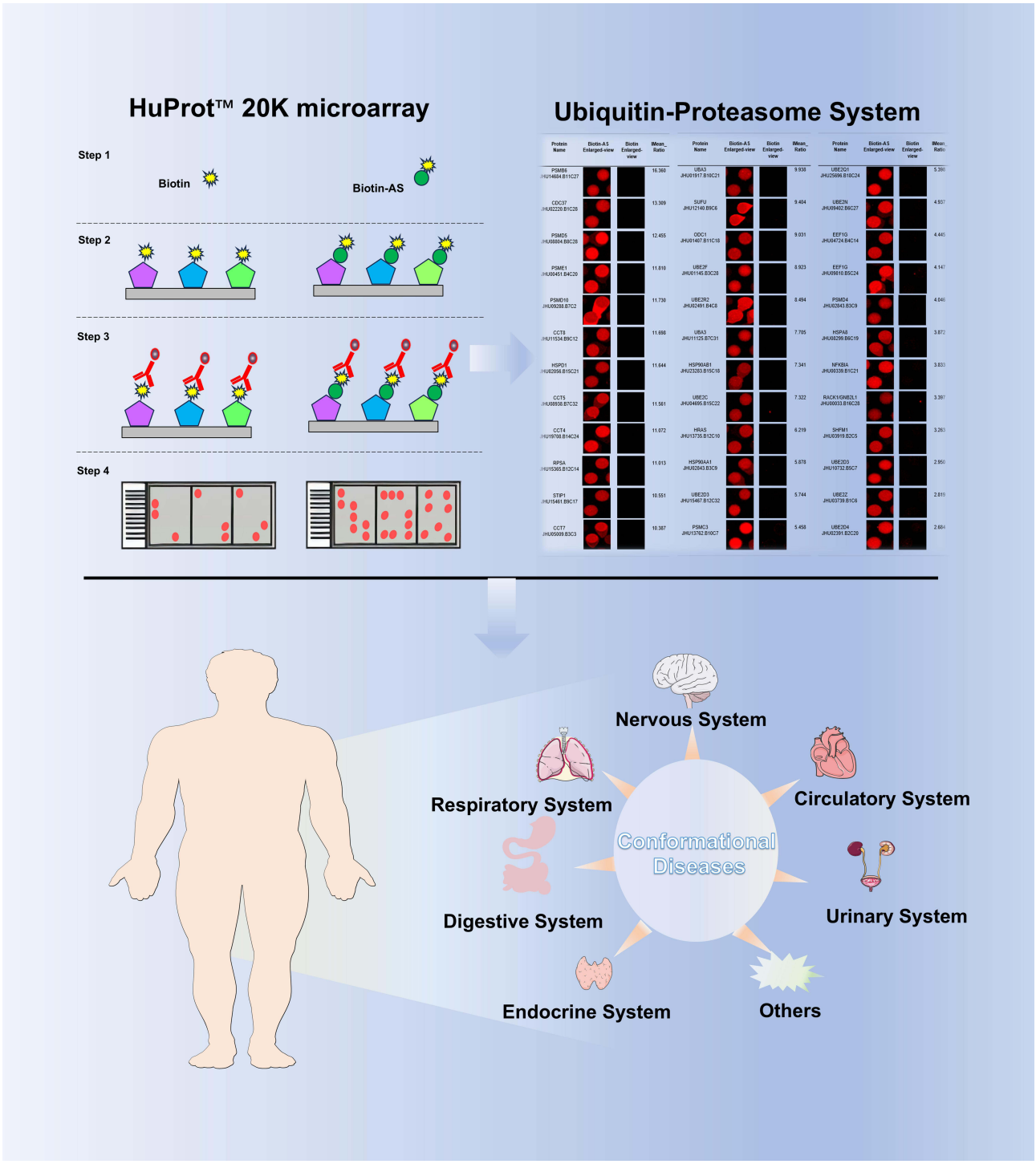
Graphical Abstract



activation.[8] Collectively, these observations pointed to the UPS as a plausible node for AS. Nevertheless, the existing data is fragmented, and full spectrum of AS-targeted proteins within the UPS is conspicuously absent.

To address this gap, HuProt™ 20K human proteome microarray, the highest throughput protein chip in the world, was employed for its ability to interrogate direct interactions between AS and thousands of individual human proteins in

a single experiment.[9,10] This approach is particularly powerful for identifying unexpected off-targets of other small molecules in prior studies successfully.[11]

By constructing this direct protein-binding profile for AS, we provided a valuable resource for elucidating its mechanism of action. Given the emerging role of AS in targeting the UPS, clarifying this interaction landscape is essential for understanding its therapeutic potential in CDs and for guiding the development of safer and more effective combination therapies.

Materials and Methods

Reagents

AS (T0433, CAS 88495-63-0) was purchased from TargetMol (USA). HuProt™ 20K human proteome microarray was obtained from the Johns Hopkins Medical Institutions Protein Microarray Core (CDI Laboratories, Inc).

Literature Search and Selection Criteria

The electronic databases PubMed and Web of Science were searched from inception until May 1, 2025. The search strategy was developed in consultation with a medical librarian and utilized a combination of Medical Subject Headings (MeSH) terms and keywords related to "artesunate", "treatment effects" and "treatment-related adverse events". Additionally, the reference lists of included articles and relevant review articles were manually screened to identify additional eligible studies.

HuProt™ 20K Human Proteome Microarray

HuProt™ 20K human proteome microarray is able to capture drug-target binding events that are difficult to detect due to the low protein concentrations in the cellular environment.[11] The assay was conducted by Wayen Biotechnologies (Shanghai, China). In brief, blocked proteome microarrays were incubated with 10 μM Biotin and 10 μM Biotin-AS for 1 h. After washing, the arrays were treated with a 0.1% Cy5-Streptavidin solution for 30 min in the dark. Signal was detected after a final wash and centrifugation step (1500 × g, 3 min) using a GenePix 4000B microarray scanner (Axon Instruments, CA).

Network Analysis

The Search Tool for Recurring Instances of Neighboring Genes (STRING) system was used to build the biological interaction networks for AS-binding proteins and first-ranked network proteins. The organism and confidence were set to "Homo sapiens", "medium-confidence" and "k-means clustering", respectively. Next, protein-protein interaction (PPI) networks were generated and then visualized by Cystoscope software. Finally, the PPI network was analyzed by MCODE, a graph-theoretic clustering algorithm. The modules in the top 4 rankings were selected for further analysis.

Results

The Global View of AS-Binding Proteins

In order to identify the binding proteins of AS, we used the proteome microarray to identify the binding proteins of AS by biotinylating AS, incubating it with the microarrays, and the binding capacity was detected with Cy5-conjugated streptavidin (Cy5-SA)[12] (Figure 1A). Therefore, we set up an experimental group (Biotin-AS) and a control group (Biotin), and Figure 1B showed the same area on the chip of the randomly selected experimental and control groups, showing that compared with negative control (green arrow), the experimental group had more positive signals (blue arrow) (Figure 1B). In the end, we identified a total of 867 proteins that directly interact with AS.

Interactions Network of AS-Binding Proteins

Next, after excluding non-specific signals, a total of 867 proteins were identified as the AS candidate binding proteins. To analyze the interaction network of AS-binding proteins completely, the above 867 candidate proteins were clustered using PPI. The top 4 ranked protein network was presented with confidence scores >0.7 and illustrated in Table 1. Among them, sorting by descending order, the first-ranked network was ubiquitin-mediated proteolysis (P_{value}=

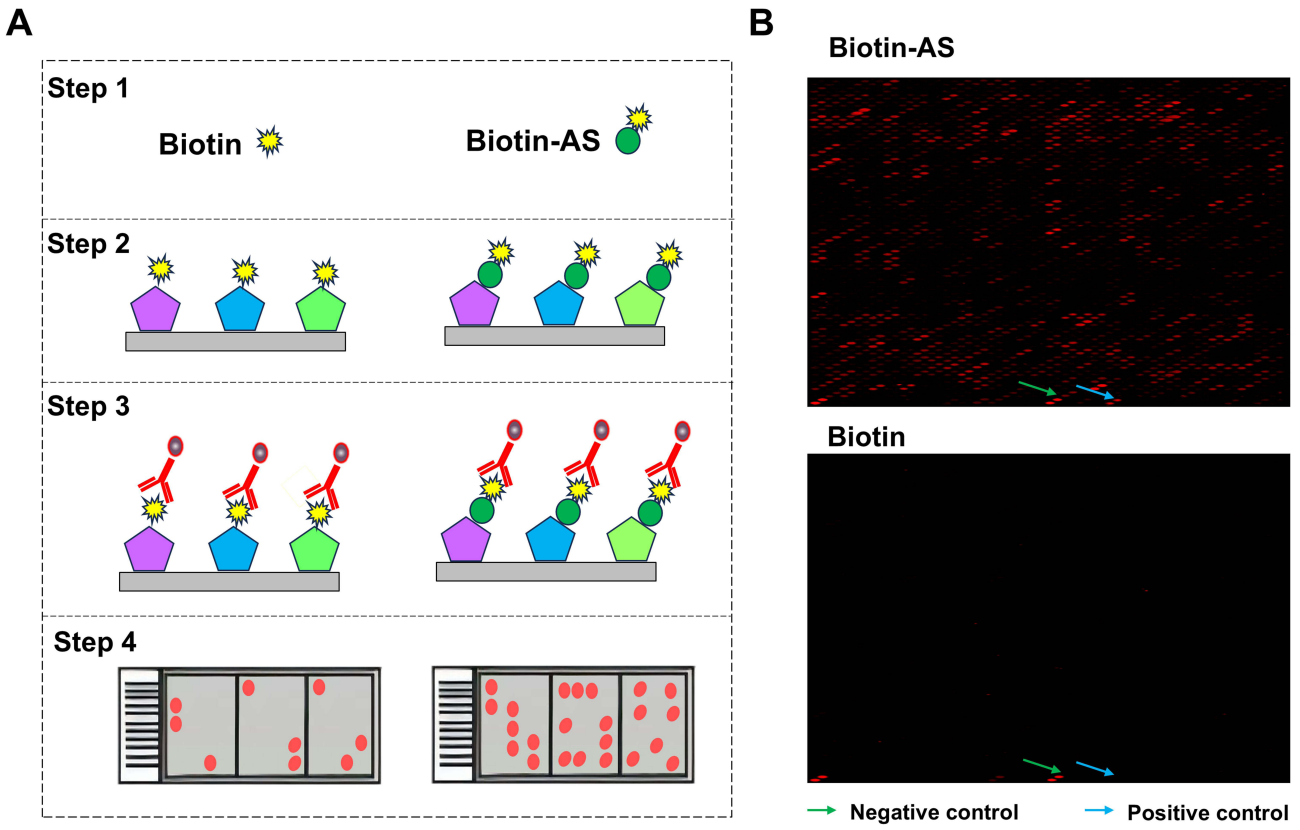


Figure 1 The global view of AS-binding proteins. (**A**) Schematic diagram of the identification of AS-binding proteins using proteome microarray. (**B**) The randomly selected Biotin and Biotin-AS were analyzed within the same area on the chip.

0.05925503, Score = 9.438), including SUFU, PSMB6, UBE2F, PSMD5, UBE2D3, PSME1, PSMD4, HSP90AA1, ODC1, HRAS, STIP1, HSP90AB1, RPSA, CDC37, EEF1G, HSPA8, UBE2Z, NFKBIA, UBE2N, PSMD10, CCT8, HSPD1, CCT5, UBE2C, UBA3, PSMC3, UBE2Q1, CCT7, UBE2R2, SHFM1, UBE2D4, GNB2L1, CCT4. The second-ranked network was the mineral absorption (P_{value}= 0.00555075, Score = 5.6), including MT1F, MT1H, MT2A, MT1E, MT1G, MT1X. The third-ranked network was the Salmonella infection (P_{value}= 8.1897E-06, Score = 5), including SNX4, DYNLT3, DYNLL1, DYNLRB2, TCTEX1D2. The fourth-ranked network was the glycolysis/gluconeogenesis (P_{value}= 0.00083301, Score = 4.872), including MAPK3, DCTPP1, HK3, NT5C2, POLD2, ADSSL1, EIF4EBP1, POLD1, RHEB, PKLR, POLA2, PFKFB3, KHK, ACTN4, GAPDH, ADSL, GCK, ACTN1, AKR1B1, STAT4, PTPN2, ALDOA, PFKFB4, RPA2, MAPK12, NT5C3A, STAT3, IFNG, SHCBP1, GMPS, ATF2, DCTD, NCAPH, MAD2L1, HK2, APRT, RRM2, PIK3R1, RPA3, HK1.

Table 1 The Four Top-Ranked Protein Network of AS-Binding Proteins

Term	P-value	Score	Genes
Ubiquitin mediated proteolysis	0.05925503	9.438	SUFU, PSMB6, UBE2F, PSMD5, UBE2D3, PSME1, PSMD4, HSP90AA1, ODC1, HRAS, STIP1, HSP90AB1, RPSA, CDC37, EEF1G, HSPA8, UBE2Z, NFKBIA, UBE2N, PSMD10, CCT8, HSPD1, CCT5, UBE2C, UBA3, PSMC3, UBE2Q1, CCT7, UBE2R2, SHFM1, UBE2D4, GNB2L1, CCT4
Mineral absorption	0.00555075	5.6	MT1F, MT1H, MT2A, MT1E, MT1G, MT1X
Salmonella infection	8.1897E-06	5	SNX4, DYNLT3, DYNLL1, DYNLRB2, TCTEX1D2
Glycolysis/ Gluconeogenesis	0.00083301	4.872	MAPK3, DCTPP1, HK3, NT5C2, POLD2, ADSSL1, EIF4EBP1, POLD1, RHEB, PKLR, POLA2, PFKFB3, KHK, ACTN4, GAPDH, ADSL, GCK, ACTN1, AKR1B1, STAT4, PTPN2, ALDOA, PFKFB4, RPA2, MAPK12, NT5C3A, STAT3, IFNG, SHCBP1, GMPS, ATF2, DCTD, NCAPH, MAD2L1, HK2, APRT, RRM2, PIK3R1, RPA3, HK1

Functional Characteristics of Ubiquitin-Mediated Proteolysis Proteins

Based on the top-ranked network, we spotlighted the ubiquitin-mediated proteolysis. Next, Gene Ontology (GO) analysis indicated that in terms of the biological process, AS-binding proteins were mainly enriched in protein folding. In terms of cellular components, AS-binding proteins were mainly enriched in proteasome complex. In terms of molecular function, AS-binding proteins were mostly enriched in ubiquitin-like protein conjugating enzyme activity (Figure 2A). Furtherly, Kyoto encyclopedia of genes and genomes (KEGG) analysis showed that AS-binding proteins were involved in ubiquitin-mediated proteolysis, prion disease and proteasome (Figure 2B). Subsequently, the clusters of orthologous groups/clusters of euKaryotic Orthologous Groups (COG/KOG) category indicated that 23 AS-binding proteins were enriched in posttranslational modification, protein turnover, chaperones (Figure 3A). Then, the subcellular localization indicated that 69.44% AS-binding protein were located on cytoplasm and 16.77% were located on nucleus (Figure 3B). Furtherly, ReactomePA analysis indicated that AS-binding proteins participated in Antigen processing: Ubiquitination & Proteasome degradation (Figure 3C). In addition, Wikipathway analysis showed that AS-binding proteins were involved in Proteasome degradation, Parkin ubiquitin proteasomal system pathway, Alzheimer's disease (Figure 3D).

Enlarged-View of Ubiquitin-Mediated Proteolysis Proteins

We represented the enlarged-view, Imean ratio value and ID number in proteome microarray for the first-ranked network proteins from experimental group (Biotin-AS) and control group (Biotin). IMean ratio value can represent the binding ability between AS and target protein. The IMean ratio of two duplicate sites was assessed to normalize the data and set to ≥1.414. According to the Imean ratio score, the highest ranked protein was PSMB6 (JHU14684.B11C27, IMean ratio = 16.360), the second ranked protein was CDC37 (JHU02220.B1C28, IMean ratio = 13.309) and the third ranked protein was PSMD5 (JHU08804.B8C28, IMean ratio = 12.455). In addition, at the end of the Imean ratio score is UBE2D4 (JHU02391.B2C20, IMean ratio = 2.684), followed by UBE2Z (JHU03739.B1C6, IMean ratio = 2.819) and then UBE2D3 (JHU10732.B5C7, IMean ratio = 2.950). Overall, compared with control group (Biotin), the experimental group (Biotin-AS) had more positive signals with twice-repeated highlighted red dots (Figure 4).

Interactions Network of Ubiquitin-Mediated Proteolysis Proteins

STRING system was used to build the biological interaction networks for first-ranked network proteins. The organism and confidence were set to "Homo sapiens", "medium-confidence" and "k-means clustering", respectively. As shown in the results of k-means clustering, the first-ranked network proteins were divided into two clusters.

Cluster 1: Chaperone Complex

Red dot represented cluster 1 which included 25 AS-binding proteins and enriched in chaperone complex (Figure 5). According to the descending order of Imean ratio score, cluster 1 included PSMB6 (JHU14684.B11C27, IMean ratio = 16.360), CDC37 (JHU02220.B1C28, IMean ratio = 13.309), PSMD5 (JHU08804.B8C28, IMean ratio = 12.455), HSPD1 (JHU02056.B15C21, IMean ratio = 11.644), CCT8 (JHU11534.B9C12, IMean ratio = 11.698), CCT5 (JHU08938.B7C32, IMean ratio = 11.561), PSME1 (JHU00451.B4C20, IMean ratio = 11.810), PSMD10 (JHU09288.B7C2, IMean ratio = 11.730), CCT4 (JHU19708.B14C24, IMean ratio = 11.072), STIP1 (JHU15461.B9C17, IMean ratio = 10.551), CCT7 (JHU05009.B3C3, IMean ratio = 10.387), RPSA (JHU15365.B12C14, IMean ratio = 11.013), SUFU (JHU12140.B9C6, IMean ratio = 9.404), ODC1 (JHU01407.B11C18, IMean ratio = 9.031), HSP90AB1 (JHU23283.B15C18, IMean ratio = 7.341), HRAS (JHU13735.B12C10, IMean ratio = 6.219), HSP90AA1 (JHU02843.B3C9, IMean ratio = 5.878), PSMC3 (JHU13762.B10C7, IMean ratio = 5.458), UBE2N (JHU09402.B6C27, IMean ratio = 4.937), EEF1G (JHU04724.B4C14, IMean ratio = 4.445), PSMD4 (JHU02843.B3C9, IMean ratio = 4.046), HSPA8 (JHU08299.B6C19, IMean ratio = 3.872), NFKBIA (JHU00339.B1C21, IMean ratio = 3.833), RACK1/GNB2L1 (JHU00033.B16C28, IMean ratio = 3.397), SHFM1 (JHU03919.B2C5, IMean ratio = 3.263).

Firstly, the molecular chaperone system consists of heat shock proteins (HSPs) and cochaperones.[13] Major HSP families include HSP110, HSP90, HSP70, HSP60 and small Heat Shock Proteins (sHSPs). HSP110 functions as a potent holdase chaperone and nucleotide exchange factor that prevents protein aggregation and regulates the HSP70 cycle. HSP90 acts at the late stage of folding and recognizes partially folded proteins and assists in their maturation or

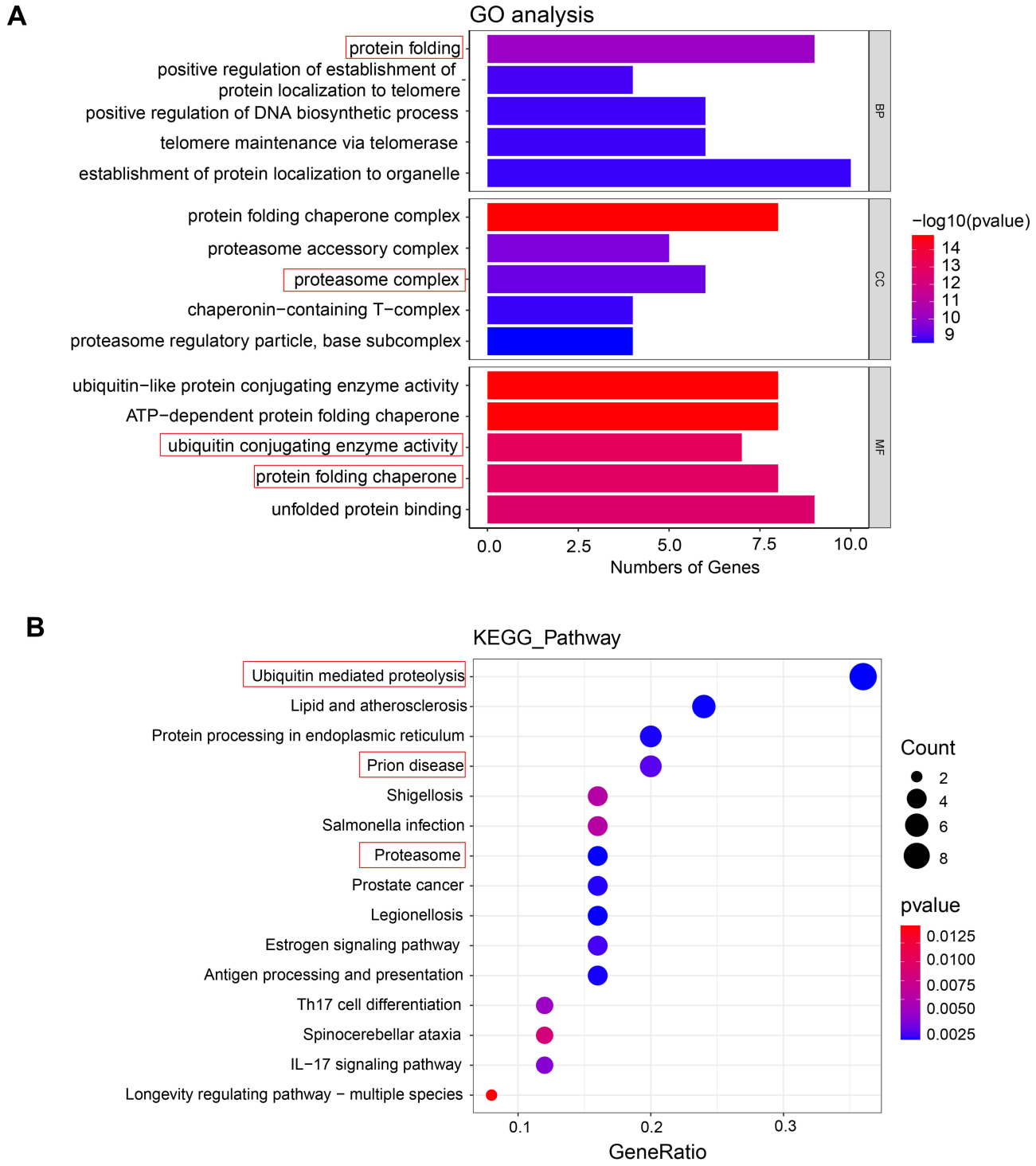


Figure 2 GO and KEGG analysis of ubiquitin-mediated proteolysis proteins. (**A**) GO analysis including biological process, cellular components, molecular function of ubiquitin mediated proteolysis proteins. Red squared box highlighted AS-binding proteins were mainly enriched in protein folding, proteasome complex, ubiquitin-like protein conjugating enzyme activity, protein folding chaperone. (**B**) KEGG analysis of ubiquitin mediated proteolysis proteins. Red squared box highlighted AS-binding proteins were involved in ubiquitin-mediated proteolysis, prion disease and proteasome.

degradation by the proteasome. HSP70 directs protein unfolding, disassembly, refolding or degradation. HSP60 acts at the early stage of protein folding and provides a closed site for protein folding.[14,15] In the context of this study, several members of the HSP system, including 60 kDa HSP, Member D1 (HSPD1), heat shock cognate 71 kDa protein (HSPA8), HSP 90 Alpha Family Class B Member 1 (HSP90AB1), HSP 90 Alpha Family Class A Member 1 (HSP90AA1), Hsp90

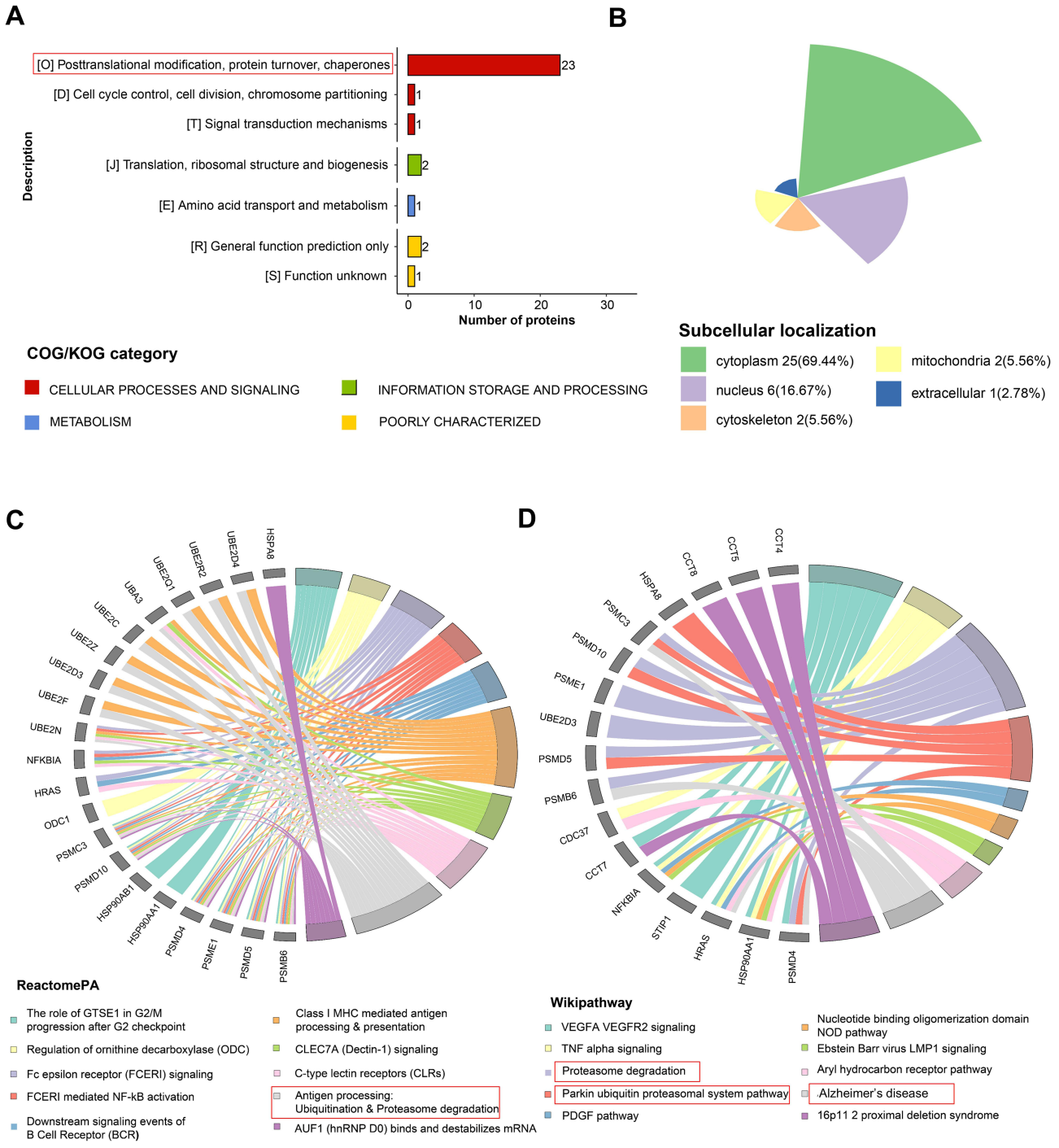


Figure 3 Subcellular, COG/KOG, ReactomePA, Wikipathway analysis of ubiquitin-mediated proteolysis proteins. (**A**) COG/KOG category analysis. Red squared box highlighted AS-binding proteins were enriched in posttranslational modification, protein turnover, chaperones. (**B**) Subcellular localization analysis. (**C**) ReactomePA analysis. Red squared box highlights AS-binding proteins participated in Antigen processing: Ubiquitination & Proteasome degradation. (**D**) Wikipathway analysis. Red squared box highlighted AS-binding proteins were involved in Proteasome degradation, Parkin ubiquitin proteasomal system pathway, Alzheimer's disease.

co-chaperone Cdc37 (CDC37), and stress-induced-phosphoprotein 1 (STIP1), have been identified as AS-binding proteins. CDC37 promoted their interaction with the Hsp90 complex, resulting in stabilization and promotion of their activity.[16] STIP1 acted as a co-chaperone for HSP90AA1 and mediated the association of the molecular chaperones HSPA8/HSC70 and HSP90.[17]

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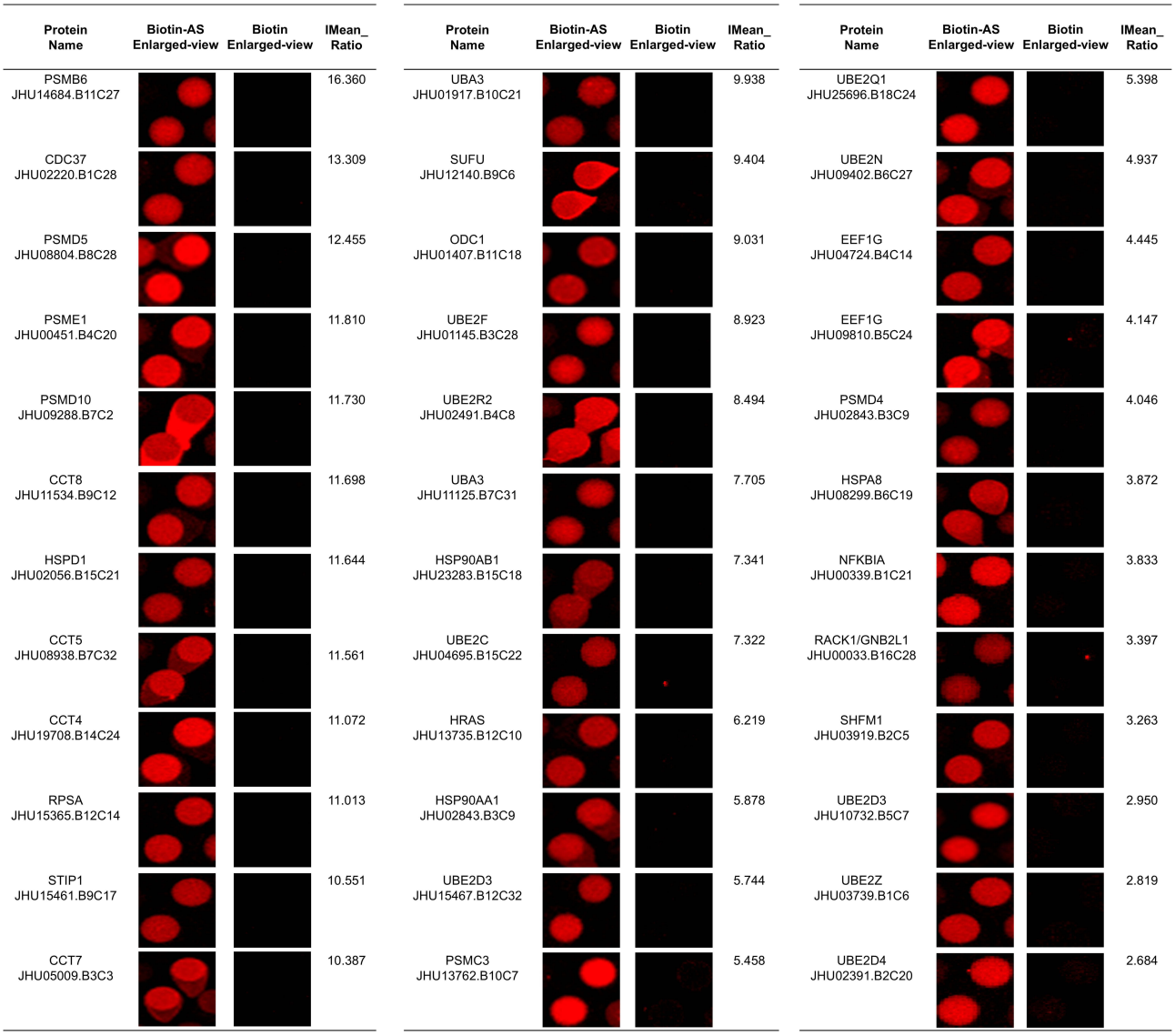

Figure 4 Enlarged-view of ubiquitin-mediated proteolysis proteins. The enlarged-view, Imean ratio value and ID number in proteome microarray were exhibited.

Then, chaperonin molecules assist protein folding, assembly, transport and degradation in the cell and play important physiological roles in DNA replication, transcription, cytoskeletal function and intracellular signalling.[18] Chaperonin containing T-complex protein 1 (CCT) is the only chaperonin molecule in the cytoplasm of eukaryotic cells and is required for the folding of approximately 15% of mammalian proteins.[19] Here, T-complex protein 1 subunit epsilon (CCT5), T-complex protein 1 subunit eta (CCT7), T-complex protein 1 subunit theta (CCT8), T-complex protein 1 subunit delta (CCT4) are AS-binding proteins.

Next, the 26S proteasome is the major protease in eukaryotic cells and is responsible for protein degradation in both the cytoplasm and nucleus. The 26S proteasomes contain a central barrel-shaped core particle (20S proteasome), which consists of four stacked seven-membered rings.[20] The proteasome is a vast complex of proteolytic enzymes, and majority (at least 80%) of protein degradation in mammalian cells is catalysed by the proteasome, including the rapid degradation of misfolded proteins as well as the slower breakdown of most cellular proteins. In recent years, proteasome inhibitors have proved to be very valuable research tools and therapeutic agents.[21,22] Here, 26S proteasome non-ATPase regulatory family (PSMD4, PSMD5, PSMD10), 26S proteasome regulatory subunit 6A (PSMC3), Human Split Hand/Foot Malformation Type 1 (SHFM1) are 26S proteasome regulatory subunit. Proteasome subunit beta type-6 (PSMB6) is

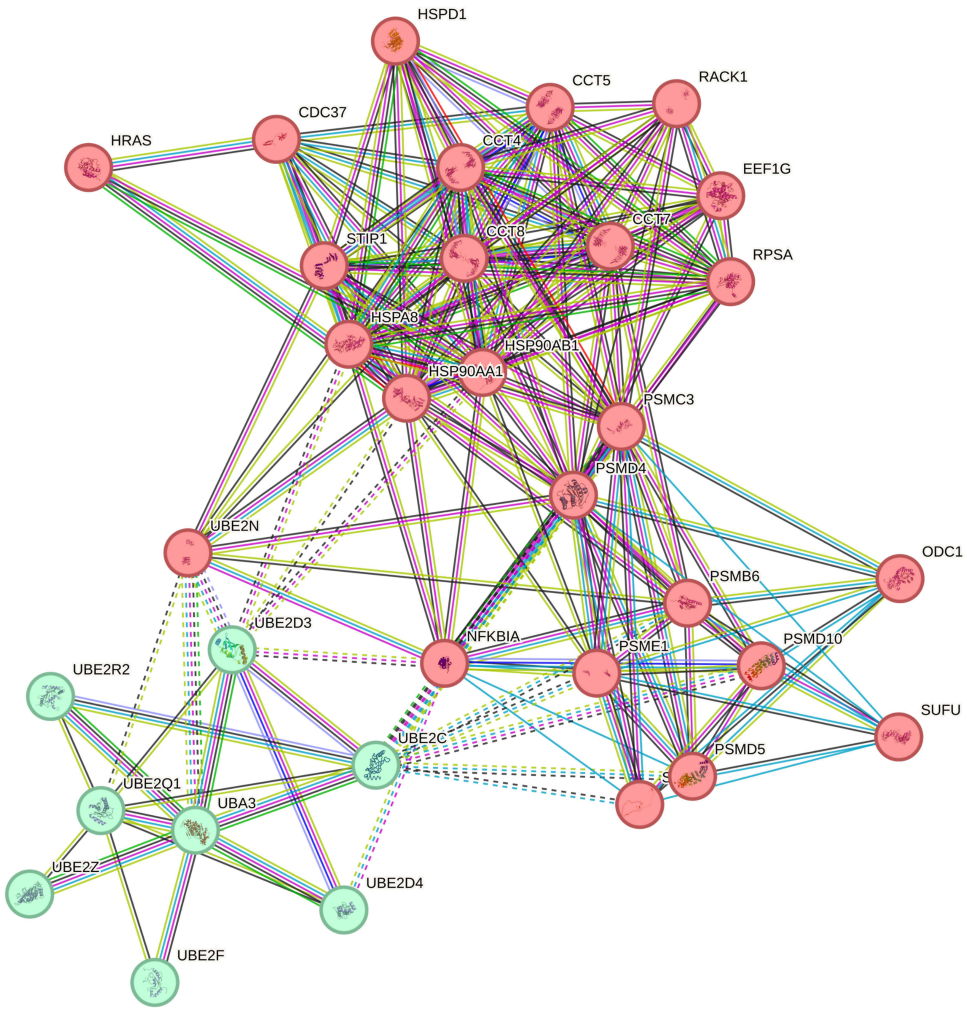

Figure 5 Interactions network of ubiquitin-mediated proteolysis proteins. Protein-protein interactions network of ubiquitin mediated proteolysis proteins. All proteins were divided into two significant protein clustering modules. Red dot represented cluster 1 which enriched in chaperone complex. Green dot represented cluster 2 which enriched in ubiquitin-like protein conjugating enzyme activity.

the component of the 20S core proteasome complex involved in the proteolytic degradation of most intracellular proteins. The 40S ribosomal protein SA (RPSA) is required for the assembly and/or stability of the 40S ribosomal subunit and the processing of the 20S rRNA-precursor to mature 18S rRNA.[23,24]

Finally, GTPase HRas (HRAS) is involved in the activation of Ras protein signal transduction. Ras proteins bind GDP/GTP and possess intrinsic GTPase activity.[25] Elongation factor 1-gamma (EEF1G) is a structural component of the eEF1 complex, playing a role in anchoring the complex to other cellular components.[26] Suppressor of fused homolog (SUFU) is part of a corepressor complex that acts on DNA-bound GLI1. SUFU also acts by linking GLI1 to BTRC and thereby targeting GLI1 to degradation by the proteasome.[27] NF-kappa-B inhibitor alpha (NFKBIA) could inhibit the activity of dimeric NF-kappa-B/REL complexes. On cellular stimulation by immune and proinflammatory responses, NFKBIA becomes phosphorylated, promoting ubiquitination and degradation.[28] Proteasome activator complex subunit 1 (PSME1) implicated in immunoproteasome assembly and required for efficient antigen processing.[29] Ubiquitin-conjugating enzyme E2 N (UBE2N) encoded a member of the E2 ubiquitin-conjugating enzyme family and played a role in DNA postreplication repair.[30] Ornithine decarboxylase 1 (ODC1) forms a complex with antizyme. The Hsp70 system then binds to this ODC1-antizyme complex, promoting its recognition and degradation by the 26S proteasome.[31] Guanine nucleotide-binding protein, beta polypeptide 2-like 1 (GNB2L1) interacts directly with the chaperonin CCT/

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TRiC complex to facilitate the folding of its specific client proteins. It also functions as a co-chaperone by organizing signaling complexes with Hsp90, thereby regulating client protein maturation and stability.[32]

Cluster 2: Ubiquitin-Like Protein Conjugating Enzyme Activity

Green dot represented cluster 2 which included 8 AS-binding proteins and enriched in ubiquitin-like protein conjugating enzyme activity (Figure 5). According to the descending order of Imean ratio score, cluster 2 included UBE2F (JHU01145.B3C28, IMean ratio = 8.923), UBE2R2 (JHU02491.B4C8, IMean ratio = 8.494), UBA3 (JHU11125. B7C31, IMean ratio = 7.705; JHU01917.B10C21, IMean ratio = 9.938), UBE2C (JHU04695.B15C22, IMean ratio = 7.322), UBE2D3 (JHU15467.B12C32, IMean ratio = 5.744; JHU10732.B5C7, IMean ratio = 2.950), UBE2Q1 (JHU25696.B18C24, IMean ratio = 5.398), UBE2Z (JHU03739.B1C6, IMean ratio = 2.819), UBE2D4 (JHU02391. B2C20, IMean ratio = 2.684).

Ubiquitin is a small molecule protein present in all eukaryotes, consisting of 76 amino acids, whose main function is to tag proteins for degradation and then use the 26S proteasome to degrade the target protein.[33,34] Ubiquitin is attached to target proteins by a series of ubiquitin-initiating enzymes, including E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin-conjugating enzyme. The degradation process begins with the activation of free ubiquitin by ubiquitin-activating enzyme E1 with the participation of adenosine triphosphate (ATP), and the formation of a thioester bond between the cysteine residue on E1 and the glycine residue at the end of the C-terminus of ubiquitin. The activated ubiquitin is transferred to the active cysteine residue of E2, forming a high-energy thioester bond. E2 then delivers ubiquitin to the corresponding E3, which directly or indirectly facilitates the transfer of ubiquitin to the target protein, where it is attached to the lysine amino group of the target protein via an isopeptide bond. Typically, target proteins can link multiple ubiquitins to form a multimeric ubiquitin chain, which is known as ubiquitination. After ubiquitin labelling, proteins can be recognised by the proteasome and degraded as substrates.[35,36] Here, ubiquitin-conjugating enzyme E2 family (UBE2R2, UBE2C, UBE2D3, UBE2Q1, UBE2Z, UBE2D4, UBE2F) accepted ubiquitin from the E1 complex and catalyzes its covalent attachment to other proteins. The NEDD8-activating enzyme E1 subunit (UBA3) activates Cullin-RING E3 ligases, thereby promoting the turnover of regulatory proteins to control processes like steroid receptor signaling and cell cycle progression.

Bioactivities of AS

The bioactivities of AS encompassed multifaceted health-promoting effects, exhibiting its significance in digestive system, respiratory system, circulatory system, nervous system, endocrine system, urinary system, motor system and other aspects. The bioactivities of the AS are described in detail in Figure 6.

Digestive System

Studies have demonstrated the protective effects of AS across various digestive disorders. Specifically, AS has been shown to modulate the NLRP3 inflammasome in non-alcoholic fatty liver disease,[37] exhibit anti-*Helicobacter pylori* activity with potential for ulcer and gastric cancer treatment,[38] alleviate ulcerative colitis via STAT6-mediated macrophage M2 polarization,[39] and serve as a safe topical option for anal lesions.[40] Animal studies demonstrate that AS, even at near-therapeutic doses, can cause mild to moderate hepatic injury associated with oxidative stress.[41] In contrast, clinical studies have not identified clear treatment-related liver toxicity, supporting a well-established, dose- and exposure-dependent hepatotoxicity profile.[42]

Respiratory System

AS demonstrates therapeutic potential for various respiratory diseases through mechanisms encompassing anti-inflammatory, anti-oxidative, and metabolic regulation. It has been shown to mitigate acute lung injury by activating the AKT/HO-1 pathways,[43] inhibit airway remodeling in COPD and asthma via the PPAR-γ/TGF-β1/Smad and MAPK pathways, respectively,[44,45] and exert anti-tumor effects in lung cancer by targeting FABP5 to induce apoptosis and by suppressing c-Myc-dependent aerobic glycolysis.[46,47]

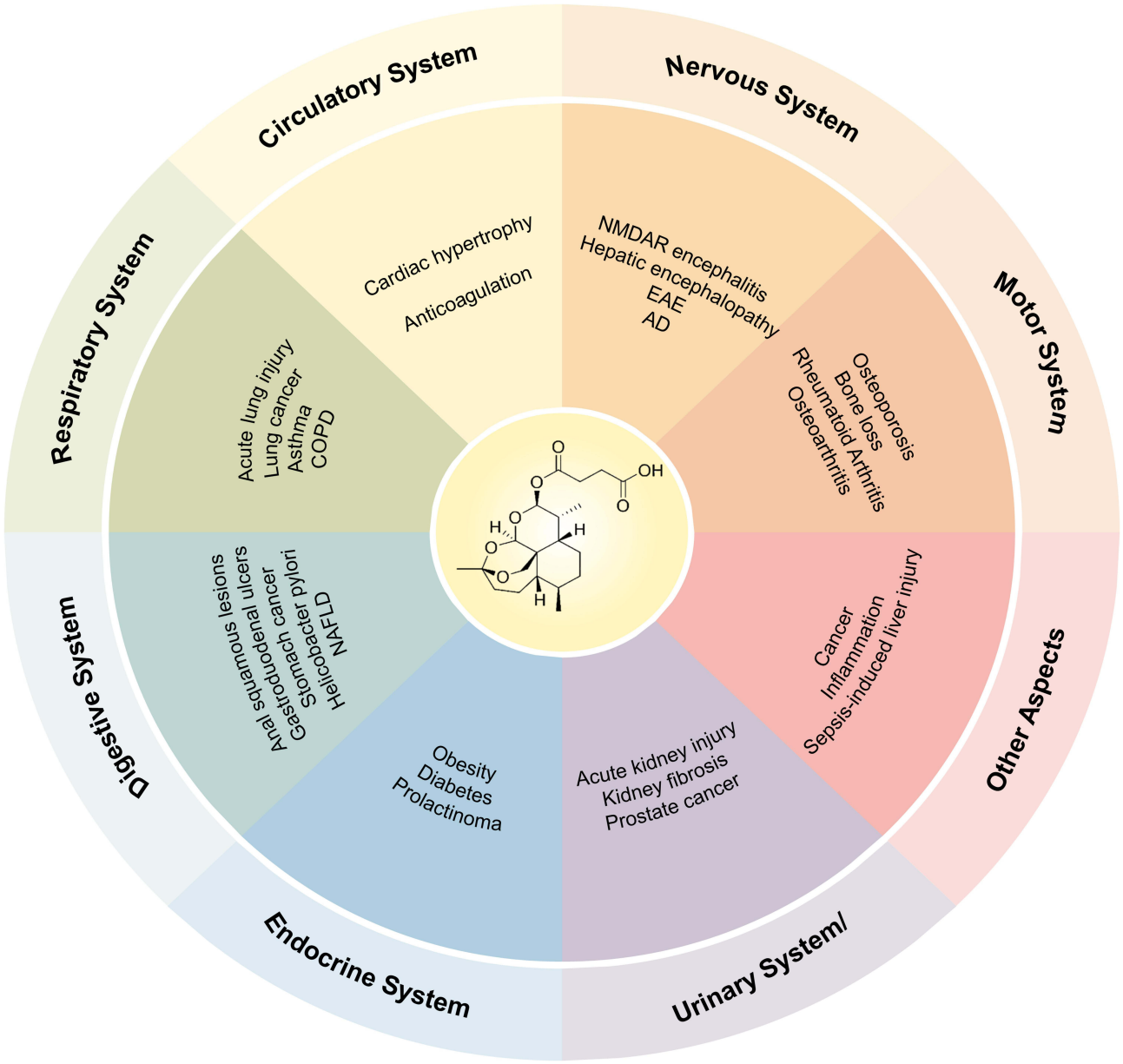

Figure 6 Bioactivities of AS. The bioactivity of AS encompasses a multifaceted range of health-promoting effects, establishing its significance in digestive system, respiratory system, circulatory system, nervous system, endocrine system, urinary system, motor system and other aspects.

Circulatory System

Recent evidence underscores the potential of AS in managing cardiovascular diseases through multi-faceted mechanisms. AS confers cardioprotection by upregulating SIRT1 and suppressing NF-κB to counteract cardiac hypertrophy.[48] It further mitigates oxidative stress by activating the Nrf2/HO-1 pathway.[49] Moreover, AS exhibits potent antiplatelet activity by inhibiting the fibrinogen-binding αIIb/β3 integrin via the cAMP pathway and suppressing the PI3K/MAPK cascade to reduce thromboxane A2 and serotonin release, thereby preventing thrombus formation.[50] Although AS shows cardioprotective effects in experimental models, these results rely on supratherapeutic doses (50–100 mg/kg), and dose-dependent differences have been reported.[51]

Nervous System

AS demonstrates significant neuroprotective potential across multiple neurological disorders. In Alzheimer's disease (AD) models, AS modulates mitochondrial dynamics, reduces Aβ deposition, attenuates neuroinflammation, and improves

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memory deficits.[52] It also restores hippocampal inhibitory glycinergic function in AD-like mice.[53] Beyond AD, AS induces PINK1/PARKIN-mediated mitophagy to counteract mitochondrial impairment in anti-NMDAR encephalitis,[54] ameliorates hepatic encephalopathy by inhibiting ammonia-induced oxidative stress and glutamate dysfunction,[55] and alleviates experimental autoimmune encephalomyelitis by blocking leukocyte CNS infiltration.[56] Notably, at high doses or with prolonged exposure, AS exhibits dose-dependent neurotoxicity, including selective brain-stem injury in mice (300 mg/kg/day orally)[57] and recent retinal toxicity at ≥10 μM in vitro or 1 mM in vivo.[58]

Endocrine System

Recent research highlights the potential of AS in treating endocrine and metabolic diseases. AS has been shown to reduce body weight and improve metabolic disorders in obesity models.[59] In diabetes, it mitigates pancreatic damage by suppressing the NLRP3/caspase-1/GSDMD pyroptosis pathway.[60] Furthermore, AS counteracts glucocorticoid-induced immunosuppression and inhibits prolactinoma growth by disrupting mitochondrial function and inducing apoptosis.[61,62] When combined with metformin, AS also alleviates diabetes-induced xerostomia by protecting salivary gland function and activating the PI3K/AKT pathway.[63] Prolonged AS exposure induces time-dependent reproductive toxicity in male rats, as evidenced by spermatogenic impairment and testicular damage.[64] In females, combination regimens or higher cumulative doses can disrupt estrous cyclicity and activate adrenal steroidogenic pathways.[65]

Urinary System

A growing body of evidence revealed that AS can modulate the immune system and glycolipid metabolism properties, suggesting an alternative for managing the urinary system and reproductive system. AS-Nanoliposome-TPP is a novel drug delivery system that targets mitochondria, mitigating acute kidney injury induced by cisplatin by suppressing oxidative stress and inflammatory responses.[66] AS was also been demonstrated repressing the growth of docetaxel-resistant prostate cancer cells.[67] Further mechanisms for protecting renal function include alleviating acute kidney injury by reducing mincle expression and inflammation in tubular epithelial cells,[68] and ameliorating diabetic kidney fibrosis through inhibition of the TGF-β-Smad signaling pathway.[69] Recent data showed that 28-day exposure to AS induced renal oxidative stress, tubular injury, and increases in BUN/creatinine, indicating a time- and dose-dependent nephrotoxicity.[70]

Motor System

AS demonstrates significant therapeutic potential for motor system disorders by targeting inflammation and bone remodeling. A novel nanosystem co-delivering AS and dexamethasone synergistically treats rheumatoid arthritis by targeting the HIF-1α/NF-κB pathway, repolarizing macrophages, and achieving targeted delivery to inflamed joints.[71] In osteoarthritis, AS mitigates cartilage damage by regulating the MTA1/LXA4 axis to inhibit the JAK2/STAT3 pathway[72] and enhancing MTA1 transcription via the USP7/FoxO1 pathway.[73] Furthermore, AS counteracts inflammation-induced bone loss by suppressing osteoclastogenesis via the TLR4/TRAF6/PLCγ1-Ca^{2+}-NFATc1 signaling cascade[74] and promotes osteogenic differentiation from bone marrow stromal cells by inhibiting the Notch1/Hes1 and NF-κB pathways.[75] General pharmacology and dog toxicology studies reported no dose-related motor impairment with AS at clinically relevant and short-term doses.[76,77]

Other System

The anticancer efficacy of AS is mediated through multiple mechanisms. It directly inhibits tumor progression by inducing apoptosis and impairing the invasion, migration, and stemness of cancer cells, such as in uveal melanoma.[78] Furthermore, AS acts as a potent sensitizer, enhancing the efficacy of both radiotherapy and chemotherapy.[79] Its anti-tumor and anti-inflammatory effects are also achieved through novel formulations; for instance, solid lipid nanoparticles containing AS can inhibit the AKT/mTOR pathway and downregulate GPX4.[80] Additionally, AS modulates the immune response by influencing macrophage polarization via pathways like lncRNA MALAT1/PTBP1/IFIH1, as demonstrated in sepsis models.[81]

Table 2 Classification and Pathogenesis of Conformational Diseases

System	Disease	Finding
Digestive System	Progressive Familial Intrahepatic Cholestasis (PFIC)	PFIC is linked to dysfunction of specific transporters, mutations in the bile salt export pump (BSEP) gene cause PFIC2, manifesting severe cholestasis.[83]
	Pancreatic Amyloidosis (PA)	Abnormal islet amyloid polypeptide (IAPP) aggregation leads to β-cell death and dysfunction, linked to type 2 diabetes and islet transplant failure.[84]
	Liver Cirrhosis	Alpha-1-antitrypsin (AAT) deficiency causes liver disease by ER retention of the mutant protein.[85]
Respiratory System	Cystic Fibrosis (CF)	Misfolding and dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein.[86]
	Alpha 1 Antitrypsin Deficiency (AATD)	The Z mutation in SERPINA1 causes AATD, where AAT misfolding and aggregation affect the liver and lungs.[87]
	Pulmonary Alveolar Proteinosis (PAP)	Pulmonary surfactant protein C (SP-C) misfolds into β-sheet structures under deacylated and neutral pH conditions, causing amyloid aggregation in PAP.[88]
Circulatory System	Light Chain Deposition Disease (LCDD)	Non-amyloid monoclonal light chain deposition in organs.[89]
	Transthyretin cardiac amyloidosis (ATTR-CA/ATTR-CM)	Misfolded transthyretin (TTR) accumulates in the myocardium, leading to cardiomyopathy and heart failure.[90]
Endocrine System	Type 2 Diabetes	IAPP aggregation forms amyloid deposits upon misfolding, leading to β-cell mass reduction.[91]
	MODY10 (INS MODY)	A2T mutation alters signal peptide structure, affecting proinsulin processing in the ER and causing MODY10.[92]
	MODY8 (CEL MODY)	A single base pair deletion in CEL causes abnormal protein tail formation, making CEL prone to aggregation.[93]
	Wolfram Syndrome (WFS)	WFS1 mutations cause cell damage and death by disrupting the function of the wolframin protein.[94]
	Medullary Thyroid Carcinoma (MTC)	RET mutations are closely associated with MTC, and somatic mutations found in sporadic cases potentially lead to abnormal calcitonin precursor protein aggregation.[95]
Nervous System	Alzheimer's Disease (AD)	AD is characterized by Aβ accumulation and aggregation of hyperphosphorylated Tau protein[96]
	Parkinson's Disease (PD)	PD pathology includes α-synuclein misfolding in Lewy bodies.[97]
	Amyotrophic Lateral Sclerosis (ALS)	ALS is closely related to TDP-43 aggregation, truncation, and phosphorylation.[98]
	Frontotemporal Lobar Degeneration (FTLD)	FTLD commonly features abnormal TDP-43 aggregation.[99]
	Huntington's Disease (HD)	CAG repeat expansion in the huntingtin gene leads to abnormal glutamine repeats in Htt that is prone to misfolding and aggregation.[100]
	Dementia with Lewy Bodies (DLB)	DLB features α-synuclein accumulation in Lewy bodies and neurites, similar to PD but with a broader distribution.[101]
	Multiple System Atrophy (MSA)	α-synuclein aggregation in oligodendrocytes forms GCIs with prion-like spread, leading to glial dysfunction, neuronal loss, and myelin autophagy.[102]
	Progressive Supranuclear Palsy (PSP)	PSP involves abnormal 4R-Tau aggregation, leading to neurofibrillary tangles and glial cell aggregate.[103]
	Corticobasal Degeneration (CBD)	CBD involves abnormal aggregation and phosphorylation of 4R Tau, affecting microtubule binding and self-assembly.[104]
	Multiple System Atrophy (MSA)	MSA is characterized by α-synuclein misfolding and accumulation in oligodendrocytes, forming GCIs, with prion-like properties.[105]
	Prion diseases	Prion diseases involve misfolding of normal PrPC to pathological PrPSc, causing neurodegeneration.[106]
	Transthyretin amyloid polyneuropathy (ATTR-PN/TTR-FAP)	ATTR-PN/TTR FAP is caused by TTR gene mutations, leading to amyloid fibril formation and deposition in nerves and organs.[107]

(*Continued*)

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Table 2 (Continued).

System	Disease	Finding
Urinary System	Diabetes related kidney diseases (DKD)	DKD involves long-term hyperglycemia causing protein misfolding and ER stress, leading to podocyte injury and albuminuria.[108]
	Glomerulonephritis	Glomerulonephritis involves immune complex deposition, potentially containing misfolded proteins, activating the complement system and causing glomerular damage.[109]
	Light Chain Deposition Disease (LCDD)	LCDD is caused by monoclonal light chain deposition in organs, causing proteinuria and renal damage.[110]
	Myeloma Kidney (MK)	MK involves tubular obstruction and injury by aggregated monoclonal light chains, causing acute renal failure and proteinuria.[111]
	Cystinosis	Cystinosis caused by CTNS gene mutations that result in the accumulation of cystine within lysosomes.[112]
	Fatal Familial Insomnia (FFI)	FFI is a rare prion disease caused by PRNP gene mutations, leading to abnormal PrPSc accumulation in neurons, causing neurodegeneration.[113]

Conformational Diseases (CDs)

CDs are a group of disorders caused by proteins that fail to fold into their native structures, leading to their improper localization/abnormal accumulation/degradation and dysfunction.[82] CDs encompass a broad spectrum of disorders in multiple systems. Table 2 described the key lesion proteins and detail of CDs.

Since misfolded proteins that accumulate in CDs are typical substrates of the UPS, UPS impairment has been identified as a key contributing factor to the pathogenesis of many CDs. Thus, targeting chaperones system and UPS might present a potential application for CDs treatment.

Discussion

AS has been extensively studied for its therapeutic potential beyond its original antimalarial application.[62,74] By integrating a novel proteome microarray identification of its direct binding proteins, we propose that AS exerts its diverse effects through a targeted modulation of the UPS, positioning it as a potential therapeutic agent for a wide range of CDs.

A common denominator of many CDs is the failure of the cellular homeostasis network, particularly the UPS, which is responsible for the selective degradation of misfolded and regulatory proteins.[36,114] When the chaperone system fails to refold a damaged protein, the UPS is activated to remove the aberrant species. Impairment at either step can lead to the toxic protein aggregation that defines CDs.[82] Therefore, therapeutic strategies aimed at bolstering this homeostasis network hold significant promise.

Our findings provide a direct molecular link between AS and this therapeutic strategy. The high-density human proteome microarray analysis identified 867 high-confidence AS-binding proteins, with the "ubiquitin-mediated proteolysis" pathway emerging as the most significantly enriched. This unbiased discovery indicated that AS directly engaged the core machinery of cellular protein quality control. We further delineated this interaction into two complementary mechanistic clusters. Cluster 1 is targeting the chaperone and proteasome system. The enrichment of AS-binding proteins like HSP90, CDC37, and components of the CCT complex and the 26S proteasome (eg, PSMD5, PSMB6) suggested that AS can directly influence the folding, stability, and ultimate degradation of a multitude of client proteins.[16,19,20] By interacting with these central hubs, AS may enhance the chaperone system's capacity to restore native protein conformation and prevent the aggregation of unfolded polypeptides. Once the chaperone and proteasome system fails to restore the three-dimensional structure of protein, the UPS were activated to remove the abnormal protein accumulation. Cluster 2 is reprogramming the ubiquitination landscape. The significant binding of AS to multiple E2 ubiquitin-conjugating enzymes (eg, UBE2C, UBE2R2, UBE2F) points to a second, potent mechanism. By interacting with these enzymes, AS has the potential to alter ubiquitination process, thereby influencing the degradation rate of specific substrate proteins that are critical in processes like apoptosis, inflammation, and cell cycle progression.[115,116,117]

This dual targeting created a compelling homeostasis-enhancing model: AS may act to promote protein refolding via chaperones firstly. For proteins beyond repair, it may facilitate their clearance by modulating the ubiquitin-proteasome cascade.[7] This hypothesis unified the diverse bioactivities of AS. For instance, its efficacy in AD models could be directly linked to enhanced clearance of Aβ and tau aggregation via boosted proteasomal activity, while its benefits in diabetic kidney fibrosis may stem from the promoted degradation of pro-fibrotic signaling proteins.[118,119]

Limitations and Future Perspectives

While this study provided the first systematic atlas of AS-binding protein, several limitations should be considered. First, the in vitro binding data from the proteome microarray may include off-target interactions whose functional relevance in a cellular or animal environment requires further validation. Second, the translational path from these identified interactions to therapeutic application faces challenges, including drug metabolism and bioavailability. Next, our proteome-wide mapping should be integrated with existing pharmacological studies. It offers a new framework that may help clarify previously reported, and potentially conflicting, mechanisms of action. Finally, the conclusions drawn in this paper are based on small-scale and preclinical studies.

Our future work will focus on the functional validation of the top-prioritized AS-binding proteins (eg, PSMB6, CDC37) in relevant cellular models using techniques, such as siRNA knockdown to confirm their mechanistic roles.[16,23] In addition, we will further evaluate the therapeutic potential of AS targeting these pathways in animal models of cancer or CDs.

Conclusion

This article performed a systematic, data-driven mapping of AS-binding protein via proteome microarray. Collectively, AS primarily functions by interacting with the UPS and chaperone complexes, positioning it as a key modulator of cellular protein homeostasis.

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Disclosure

We declared that we had no commercial or associative interest that represented a conflict of interest in connection with the work submitted.

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