Probing the molecular choreography: a chemical biology exploration of the Ubiquitin and Ubiquitin-like post-translational machinery

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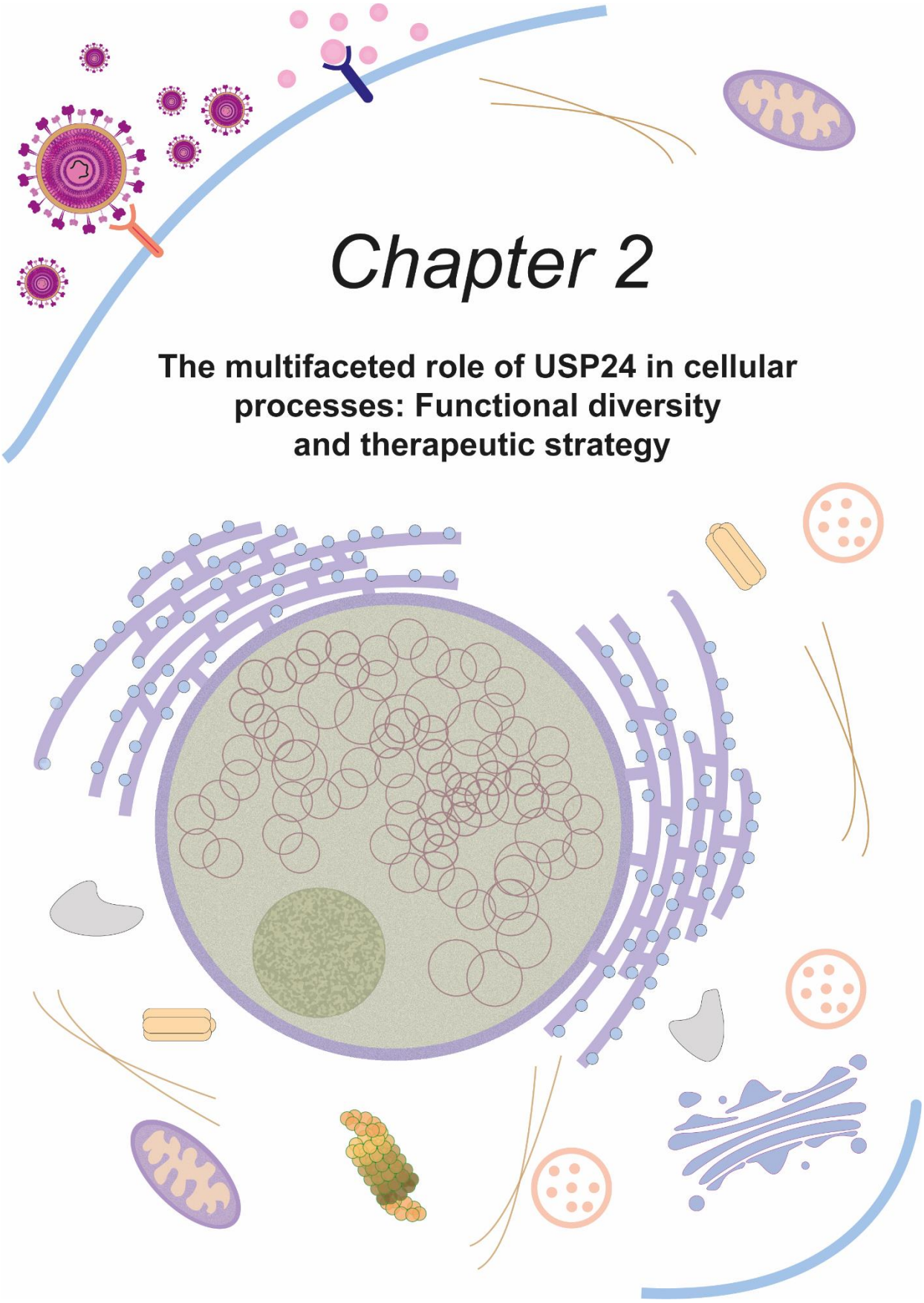

Chapter 2

The multifaceted role of USP24 in cellular processes: Functional diversity and therapeutic strategy

Abstract:

Ubiquitin functions as a post-translational modifier regulating the stability, function, localization, or conformation of modified proteins. Deubiquitinases (DUBs), a class of proteases, counteract this modification by removing ubiquitin from the substrate proteins. Among these, USP24, a member of the cysteine protease DUB family, remains relatively understudied. However, emerging evidence on its multifaceted role in cytokine regulation and immune response, DNA damage response, and genome stability implicates USP24 in cancer pathogenesis and neurodegenerative diseases and supports the importance of dissecting its druggability. Despite structural and biochemical challenges, two first-line inhibitors have been identified for USP24. However, a comprehensive understanding of their mechanism of action remains elusive. This review highlights the biology, substrates, and inhibitors of USP24, with further discussion on drug repurposing strategies that can aid in the discovery of USP24 inhibitors.

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Introduction

Ubiquitin (Ub) is a highly conserved post-translational modifier that plays a major role in protein regulation. It is a small protein of 76 amino acids that is conjugated to substrate proteins via an (iso)peptide bond by the collective action of E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 (Ub-ligase) enzymes.[1] Substrate proteins can be modified by different linkage Ub types, including monoubiquitination, a chain of Ub's called polyubiquitination via any of the seven distinct lysine residues (K6, K11, K27, K29, K33, K48 and K63) or the N-terminal methionine of Ub. The fate of the substrate protein is decided based on the linkage types. [1-2]

Ubiquitination can be reversed by a specific group of proteases called deubiquitinases (DUBs).[3] DUBs have been found to have various biological substrates, playing a crucial role in physiological events. Depending upon the substrate and the specificity of the DUB towards mono- or poly-Ub chains and towards particular Ub-linkage (K48, K63, etc.), they can contribute to a number of biological events. Therefore, their dysregulation has been observed in many human ailments.[2] [4] So far, around 100 DUBs have been identified to be encoded by the human genome.[5] DUBs can be classified into seven groups based on their catalytic mechanism and structure, which include Ubiquitin-Specific Proteases (USPs), Ubiquitin Carboxy-terminal Hydrolases (UCHs), Ovarian Tumor Proteases (OTUs), Josephine and JAB1/MON/MOV34 (JAMM) as well as recently identified MINDY[6] and ZUFSP/ZUP1[7] families. All DUB families, except for the JAMM family, are cysteine proteases that share a common catalytic mechanism and consist of a catalytic cysteine residue in a catalytic dyad or triad similar to Papain proteases.[8]

USP24 is one of the largest members of the USP family of DUBs, was initially found to be located at the *PARK10* locus of the human genome associated with late-onset Parkinson's disease, [2, 9] but was later found to be involved in various DNA damage pathways,[10] cancer metastasis,[11] infectious diseases[12] and neurodegenerative disorders[13] which makes it an attractive druggable target. Here, we will delve into the biological importance of USP24 among other DUBs and summarize all the literature on USP24 published so far, providing future investigators with a comprehensive review of this DUB. Further, we will address the structural architecture of USP24 and its resemblance with other homologous DUBs. In addition, we will translate our understanding of USP24 biology and structure towards inhibitor development. Here, we will critically analyze inhibitors already reported for USP24 and describe the challenges and scope of advancement for future inhibitor discovery ventures.

The structural architecture of USP24

USP24 is a multidomain protein of 2,620 amino acids, of which very little structural information is known. Structural prediction programs have only identified its name-giving catalytic domain (CD) towards the C-terminus as well as a single ubiquitin-associated domain (UBA) near the very N-terminus (Figure 1A). [11] For all other regions, no conclusive domain identifications were made, nor were any USP24 domains experimentally validated. Without experimental structural information, scientists resort to homology modeling[14] or structure predictors such as RoseTTAFold[15] or AlphaFold.[16] Homology modeling of the USP24 catalytic domain identified USP7[11] as a closely related DUB, further identifying the catalytic residues. Although residues C1698 and H1970 were found, and the active site cysteine was experimentally validated,[17] A third catalytic residue could not be conclusively determined yet (Figure 1B).

The AlphaFold model of USP24 identifies not only the aforementioned UBA and catalytic domain but also many repetitive α-hairpins (Figure 1A). Together, these α-hairpin repeats form a solenoid structure, known as Armadillo repeats,[18] a conserved motif[18b] not only found in the Armadillo containing repeat (ARMC) protein family but also β-Catenin, SYS-1, Importin-α, and others. [19] Proteins containing these superhelical repetitive α-hairpins have previously been reported to function in signal transduction,[20] mitochondrial function regulation,[21] tumor progression,[22] and embryonic development,[19, 23] where these superhelical repeats aid in diverse protein-protein and protein-peptide interactions through the concave interacting surface. Since USP24 has similar motifs present and given its reported function in DNA damage response,[10, 24] tumor progression,[11, 25] cytokine regulation[25a, 26] and regulation of mitochondrial function, this intrigues further understanding of the structural properties of USP24, which can advance our ability to target it in disease contexts and pave the way for future investigations into its physiological and pathological functions, which will thoroughly be discussed in the following sections of this review.

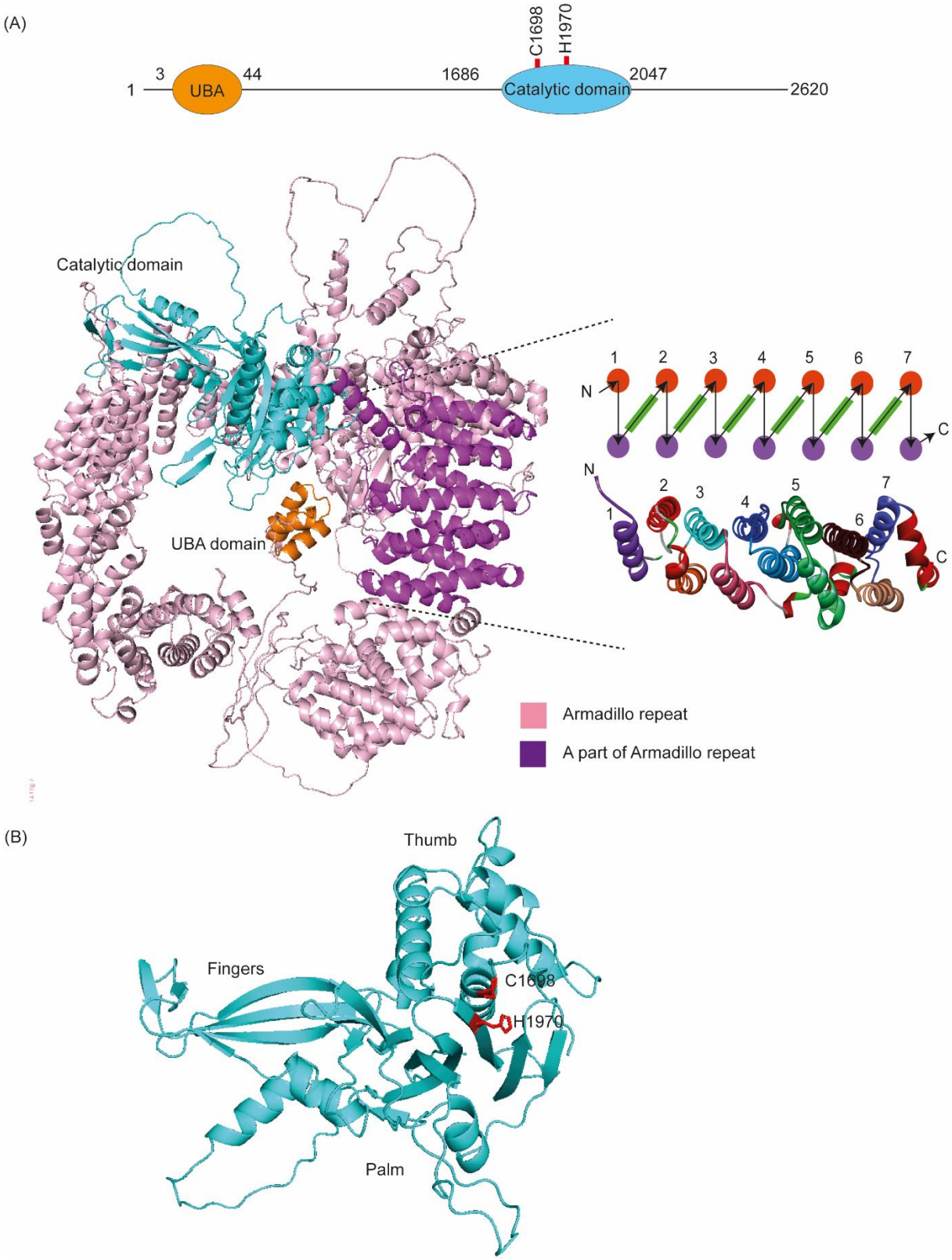

Figure 1: Structure of USP24. (A) *Above: Schematic representation of USP24 with the active cysteine (C1698) and histidine (H1970) in red. Below: Full-length structure of USP24 (AlphaFold) highlighting the catalytic domain (Cyan) and the UBA domain (Orange). Armadillo repeats are shown in light pink.*

It also highlights one arm of the characteristic Armadillo domain (Magenta): A classical Armadillo repeat arrangement, showing how alpha helices are arranged as hairpin repeating units both as a schematic diagram and cartoon representation. (B) *The catalytic domain of USP24 depicts a classical right-handed conformation. The two catalytic residues identified (C1698 and H1970) for USP24 have been highlighted in red.*

Diving into the functional diversity of USP24

USP24 is found to be involved in many cellular pathways, including cell proliferation, apoptosis, DNA damage response, genomic stability, and gene expression. Its deubiquitinase function is associated with neurological diseases and different types of cancers.[27] But USP24 is much more than just a large DUB. Recently, our group reported its role as an ISG15 cross-reactive DUB, demonstrating the importance of USP24 in regulating innate immune response.[28] This has opened up a new avenue to look at DUBs and their cross-reactivity with Ub-like modifiers. This section will discuss the function of USP24 in diverse biological pathways and how it regulates these functions by targeting its ubiquitinated and ISGylated substrates.

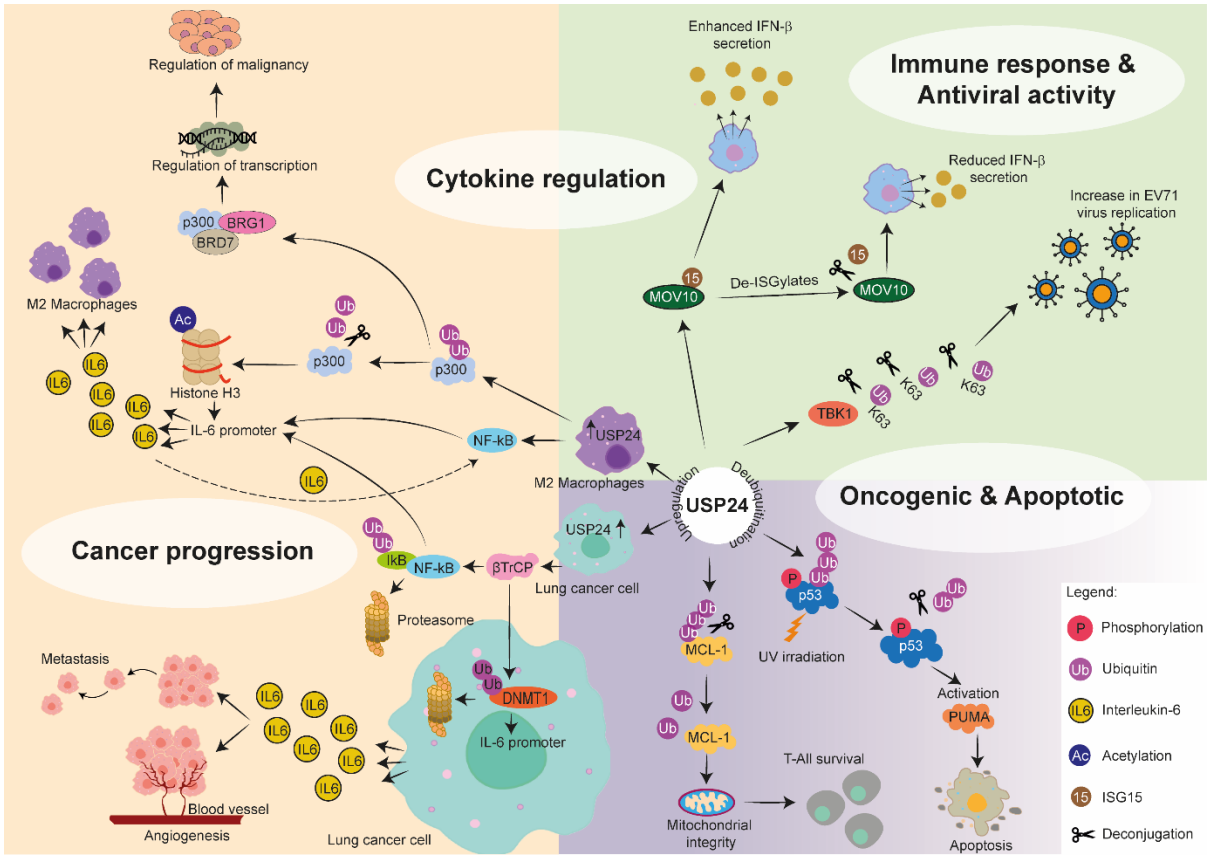

Figure 2: Biological roles of USP24: *Schematic overview highlighting the broad range of biological effects that USP24 shows and how it can be crucial in downstream pathways. Towards the right side is the deubiquitination and recently identified de-ISGylation effects of USP24, while towards the left, it mostly highlights the overexpression of the enzyme. However, the final impact is via the enzymatic activity. The figure also highlights the importance of USP24 in the context of cytokine pathways (IL6 and IFNβ), cell survival, DNA damage response, tumorigenesis, and apoptosis.*

USP24 is linked to neurological disorders:

Reversible ubiquitination is a pivotal regulation mechanism for protein homeostasis. Dysregulation of protein homeostasis is the fundamental mechanism in many neurological disorders.[29] [30] Mutations or up/down-regulations of DUBs have been implicated as critical modulators in neurological disease, as they are fine-tuning regulators of ubiquitination and protein homeostasis.[31] The role of DUBs in neuronal diseases has been commonly identified.[32] [33] USP24 was initially identified as one of the potential genes at the PARK10 locus linked to Parkinson's disease (PD).[9b] [9c] [9d] [9e] Recent studies have shown its role as a negative regulator of autophagy in PD by affecting the stability of ULK1.[13] Involvement of USP24 in neurological disorders extends to Autism Spectrum Disorder (ASD), a complex neurodevelopmental genetic disorder, by identifying a significant relationship between

Autism Spectrum Disorder (ASD) and hypomethylation due to rare genetic variants (meSNVs) at the *PARK10* locus, where USP24 is one of the genes along with other loci (ERMN, METTL21C, PDE10A, STX16, and DBT) in a patient data set.[34] The multifaceted role of USP24 at the genomic level encourages further investigation of its function from a genome stability and neurological perspective. Notwithstanding the evidence of its implication in neurological disorders, the intricate mechanisms underlying this phenomenon have yet to be fully identified. A thorough understanding of these mechanisms could offer valuable insight for developing potential therapeutic strategies to combat neurological disorders.

Dual Role of USP24 as Tumor Suppressor and Oncogene

DUBs, as the key determinant of reversible ubiquitination, regulate various pathways implicated in cancer, including but not limited to cell cycle progression, cell proliferation, apoptosis, and metabolic pathways, by targeting numerous substrates within the cell. Consequently, the changes in protein levels and activities of DUBs, as well as the impact of their actions on substrate functions, dictate whether they function as tumor suppressors or oncogenes.

USP24 is one of the DUBs with a dual role in tumorigenesis and cancer progression. The protein level of USP24 undergoes reduction due to phosphorylation mediated by EGF- or CDK1-mediated at multiple sites, including Ser1616, Ser 2047, and Ser 2604. This diminished expression of USP24 correlates with early-stage cancer formation and facilitates cell cycle progression. Knockdown of USP24 leads to a decrease in Bax and p300, thereby impeding apoptosis while also reducing E2F4 and Securin, which promotes cell cycle progression. These findings suggest a potential tumor-suppressor role for USP24 in cancer progression.[17] Although the downregulation of USP24 is linked to early-stage lung cancer progression, a correlation between high levels of USP24 and late-stage lung cancer patients is indicated, [35] implying the dual function of USP24 as a tumor-suppressor or oncogene at a distinct stage of lung cancer. Recent studies persist in providing new cues on USP24 function in different types of cancers. USP24 is located in the genomic region deleted in neuroblastoma, implying that USP24 may play a critical role as a tumor-suppressor in the pathogenesis of this aggressive childhood diseases[36] while acting as an oncogene in bladder cancer and gastric carcinoma via stabilizing the oncogenes Gasdermin B (GSDMB) and PLK1, respectively.[37] [38]

The role of USP24 also extends to T-cell Lymphoblastic Leukemia (T-ALL), an aggressive form of cancer that occurs from T-cell derived neoplasms, where an alarming increase in severity has been observed since 2003.[39] USP24 plays a vital role in T-ALL persistence by deubiquitinating MCL-1 (Induced Myeloid Leukemia cell differentiation protein). MCL-1 is a BCL-2 family protein localized in Mitochondria. It is a regulator of apoptosis and maintains cell viability.[40] USP24 protects MCL-1 from proteasomal degradation and aids to the maintenance of mitochondrial integrity both in cells and

neoplasms (Figure 2).[41] [42] Inhibition of USP24 with small molecule inhibitors like WP1130 has been reported to promote apoptosis by enhancing mitochondrial membrane potential via effecting the deubiquitination of MCL-1, holding a potential as a therapeutic agent. However, further investigation is needed due to the inhibition of multiple DUBs by WP1130. The multifaceted role of USP24 in cancer biology, including its involvement in T-ALL, underscores its significance as a potential target for therapeutic intervention and highlights the need for continued research in this area.

The Roles of USP24 as Guardian of DNA Damage Response, Genomic Stability, and Gene Expression:

USP24 is crucial in several critical cellular processes, including DNA damage response, genomic stability, and gene expression. One notable aspect of its involvement is in stabilizing DDB2, a crucial component of the UV-DNA Damage Binding protein complex responsible for repairing DNA damage caused by UV radiation. DDB1-CRL4^{DDB2} E3 ligase complex ubiquitinates Damage-specific DNA-binding protein 2 (DDB2), leading to its proteasomal degradation. USP24 has been found to stabilize DDB2 by deubiquitinating and rescuing it from proteasomal degradation.[9a, 43]. However, the effect of USP24-mediated stabilization of DDB2 on DNA damage response remains to be determined. USP24 has also emerged as a key regulator of p53, another DNA damage response protein that activates DNA repair proteins, arrests cell growth by holding the cell cycle upon sensing DNA Damage, and initiates apoptosis if DNA Damage is irreversible.[44] The activity of USP24 is shown to be essential for p53 stability and activity. As a result of USP24-dependent stabilization of p53, activation of pro-apoptotic protein PUMA occurs and leads to rapid induction of apoptosis after UV irradiation (Figure 2).[24a] Notably, depletion of USP24 has implications for increased mutation rates at the *HPRT* (hypoxanthine-guanine phosphoribosyltransferase) locus, suggesting a role of USP24 in genomic stability[24a, 45]. USP24 has also been identified to be a chromosome instability gene that is deleted in neuroblastoma [36]. In murine models, the deletion of USP24 results in the degradation of Collapsin Response Mediator Protein 2 (CRMP2), leading to spindle formation defects, mis-segregation of chromosomes, and aneuploidy. Restoring CRMP2 expression rescues these phenotypes, highlighting once again the significance of USP24 in maintaining genome stability.[36, 46]

USP24 stabilizes Bromodomain (BRD) containing proteins,[47] which are essential for the regulation of gene expression via chromatin remodeling. The interaction between USP24 and Bromodomain (BRD)-containing proteins was first identified in a yeast two-hybrid assay, revealing the recruitment of numerous proteins by the C-terminal end of USP24. Knockdown of USP24 in H1299 lung cancer cells and U2OS bone cancer cells led to decreased levels of BRD-containing proteins, like BRG1, BRD7, BRD1, BRD3, GCN5, PCAF, and TIF1α. Overexpression of USP24 stabilized BRG1 and BRD7 but did not affect

their mRNA levels indicating the role of USP24 at the protein level but not at the transcription level.[47] BRG1 and BRD7 are parts of the core SWI/SNF, an ATP-dependent chromatin remodeling complex recruited in Embryonic stem cells for gene activation and repression.[48] USP24 affecting the levels of these proteins can be important globally for downstream gene regulation via chromatin remodeling. On the other hand, overexpression of BRG1 increased USP24 protein levels, indicating a reciprocal regulatory relationship between USP24 and BRG1. The interaction of USP24 and BRD7 was also explored and USP24 was reported to stabilize the protein levels of BRD7. The relevance of the USP24-BRD7 axis was further evaluated in a lung cancer mouse model. With higher levels of USP24, levels of its substrates like p53,[49] acetyl p53,[50] p300,[51] BRD7, Bax,[52] and p21[53] were also increased. All these substrates have already been reported to regulate cancer progression. For further validation, 15 lung cancer patients were examined. Patients with higher USP24 levels showed higher BRD7 levels, implying the regulation of BRD- containing proteins by USP24. This highlights the importance of USP24 and intrigues to investigate how USP24 might affect the chromatin remodeling and if there are other proteins in the chromatin remodeling complex that are affected similarly by USP24. If so, then what is the role of USP24 globally in gene regulation? Also, the interactions of USP24 with BRD7 and BRG1 were investigated, but is there any biologically important relationship between USP24 and other BRD-containing proteins reported in the paper, like BRD1, BRD3, GCN5, PCAF and TIF1α? Answering these queries might reveal new mechanisms involved in gene regulation and cancer progression.

Cross-reactive USP24 regulates cytokine pathways impacting cancer progression and antiviral response:

Cytokines, small signaling proteins, are produced and released by many cells, predominantly macrophages and helper T-cells. Cytokines are vital regulators of the immune response against infection, inflammation, and cancer.[54] They are divided into different groups, including interleukins (ILs), interferons (IFNs), and tumor necrosis factors (TNFs). Production and secretion of cytokines, as well as cytokine-mediated signaling, are tightly regulated by reversible ubiquitination and thereby, enzymes of the ubiquitin system.[55] [56] USP24 has been indicated to induce interleukin-6 (IL-6) expression by stabilizing p300 and subsequently increasing recruitment of NF-κB on DNA and Histone H3 acetylation in macrophages (Figure 2). Further, USP24 promotes nuclear translocation of NF-κB and decreases IL-6 promoter methylation by stabilizing p300 and β-TrCP, resulting in the upregulation of IL-6. As a result of regulating IL-6 levels in tumor-associated microenvironment, USP24 contributes to lung cancer malignancy.[25a]

The role of USP24 on cytokine pathways is further extended to regulation of antiviral immunity. A study has reported that USP24 promotes enterovirus 71 (EV71) infection by impeding K63-linked

polyubiquitination of TBK1. Depletion of USP24 increases K63-linked polyubiquitination of TBK1 and promotes the phosphorylation and translocation of Interferon Regulatory Factor – 3 (IRF3), leading to increased production of type I interferon (IFN-I) during EV71 infection.[57] Furthermore, our group has recently identified USP24 as an ISG15 cross-reactive DUB. This discovery adds another interesting layer to the role of USP24 in the modulation of antiviral immunity since the ISG15 level is highly regulated by a type I interferon, and it functions as cytokines. We reported that USP24 specifically deISGylates MOV10 at K121, K714 and/or K715 as the major ISG15 conjugated sites.[28] MOV10 (Moloney Leukemia Virus 10 protein) is an RNA helicase,[58] that promotes Type I Interferon production in cells and also blocks viral replication.[59] The mechanism of MOV10 regulation in modulating Type I IFN pathway was unknown. Our study identified USP24 deISGylating MOV10 to limit its function, thus blocking Type I Interferon production and subsequently, facilitating viral replication. Overall, USP24 is a negative regulator of human antiviral immune response. Thus, inhibiting USP24-mediated de-ISGylation can augment the effect of antiviral therapeutics. Also, it is worth investigating other ubiquitinated and/or ISGylated substrates of USP24 and exploring their biological function. Taken together, this discovery not only enriched the DUB narratives but also highlighted the importance of looking at cross-reactive DUBs for therapeutic interventions.

Targeting USP24: Challenges and advances

USP24 has a wide impact on various biological pathways, as outlined above. Both its increased levels and deubiquitinating activity have been associated with various human diseases,[25a, 47] which highlights its importance for therapeutic interventions. USP24 is an emerging drug target but also comes with some challenges. Here, we will discuss those challenges, advances made so far, and future scope for USP24 inhibition by drug repurposing.

Challenges: The development of USP24 inhibitors presents many challenges. Firstly, there is a lack of structural information. USP24 is a relatively big 298 kDa protein of 2,620 amino acids. So far, its structure has not been solved, and very little is known about the different structural domains. Few computational mappings and some cell-based experiments have predicted a ubiquitin-associated (UBA) domain at the N-terminus and a catalytic domain towards the C-terminus. AlphaFold predictions are in agreement with this. Hence, structure-based inhibitor design is still at the computational level. Secondly, the function of USP24 is still not very well characterized. Being a cysteine protease, it is

found to have a deubiquitinating activity, but its association with other biological pathways especially, its cross-reactivity with ISG15, is still very implicative. Thus, investigating the inhibitor effect can be challenging as well. Classical high-throughput screening efforts that rely on monitoring DUB activity are possible but will require sufficient amounts of active recombinant protein, which is challenging due to problems with its large size and instability during expression and purification and limited commercial availability. Cell-based screening brings in another possibility. With a known substrate for USP24, one could set up FRET assays in cells.[60]

Advances:

Despite the number of challenges, USP24 attracted the interest of drug developers when it was identified as modulating so many biological events, including cancer metastasis and angiogenesis.[25a] The fact that USP24 is closely related to DUBs like USP7[24a] and USP9X,[41, 61] which have been shown to play crucial roles in several cell signaling pathways, raises the possibility of USP24 being involved in similar pathways. This further substantiates the growing interest in investigating USP24 as a drug target. In that respect, any knowledge obtained regarding the structure and inhibitors of USP7, USP9x, or other closely related DUBs could be translated to USP24. Indeed, this strategy was successfully applied to identify USP24 inhibitors, as discussed below:

So far, only two compounds have been reported to inhibit USP24, which are WP1130,[41] and NCI677397 (Figure 3).[11] This part of the review will describe these inhibitors and try to understand what kind of hit compounds can be implicated as USP24 inhibitors for future drug development ventures.

WP1130 (Trade name: Degrasyn) was initially reported to downregulate Bcr/Abl, a JAK2 transducer.[62] The DUB inhibitory potential of WP1130 was first demonstrated for USP5, UCH-L1, USP9x, USP14, and USP37.[63] In fact, potential USP24 inhibition by WP1130 was reported more recently in a study in which USP24 was shown to promote T-ALL cell survival, and WP1130 treatment induced apoptosis by decreasing cell viability.[41] This study claims that WP1130 induces apoptosis by affecting the mitochondrial integrity via the USP24-Mcl-1 axis. However, a detailed mechanism of this drug action is lacking. Figure 3A shows the predicted interaction model of WP1130 in the USP24 catalytic pocket. The ligand interacts with the USP24 catalytic pocket mostly via hydrogen bonds and Van der Waals interactions. It is expected to form an irreversible covalent bond with the catalytic cysteine residue of USP24, as claimed in the literature.[41]

Perazine analog **NCI677397** was found by structure-based virtual screening using a USP7-based modeled structure of USP24, after which it was validated to inhibit USP24 deubiquitinating activity specifically amongst USP7, USP9x and USP10.[11] Molecular docking revealed the possible interactions

of NCI677397 with USP24 (Figure 3B) and provided some structural insights into its specificity for USP24 over USP7. In the same study, it was shown that NCI677397 inhibited chemotherapy-induced drug resistance in several cancer cell lines and that this could be linked to loss of USP24 activity, but a thorough investigation remains to be done.

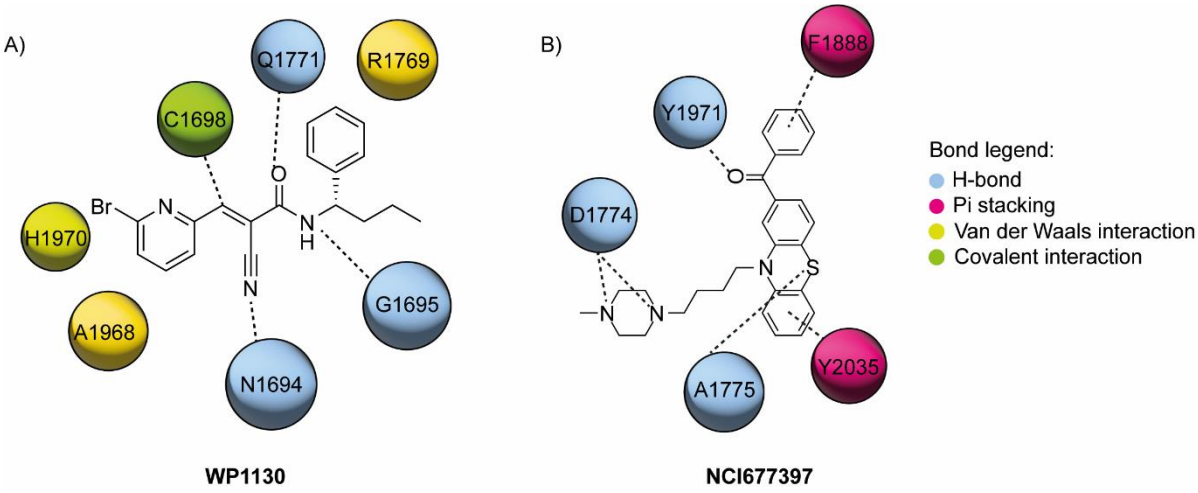

Figure 3: Targeting USP24: (A) *WP1130 interacts with the catalytic domain of USP24. The ligand engages USP24* mostly with H-bonds and a covalent interaction with C1698 (active cysteine).* (B) *NCI677397 interacts with the catalytic domain of USP24*. The aromatic rings play the major role in engaging USP24 via Pi-stacking interaction supported by H-bonds as depicted.*

**amino acid numbers correspond to the full length sequence*

Concluding remarks, opportunities and future perspectives:

USP24 presents a profound impact on various biological pathways encompassing diverse roles from cytokine modulation to cell survival. Its association with chromosome instability,[46] antiviral defense mechanism,[57] DNA damage response,[24a] and Parkinson's disease[2, 9, 64] makes it an increasingly interesting DUB target.

However, there is still much to learn about this large cysteine protease, and its structural complexity might harbor other interesting activities beyond its deubiquitinase function. A comprehensive investigation with different domain constructs of USP24 is essential to dissect its systematic structure-function relationship. Besides, a profound investigation is necessary to identify more substrates targeted by USP24 beyond the reported ones **(see Outstanding Questions).**

One of the major limitations to the biochemical investigation of USP24 is the lack of its crystal structure. Since USP24 is a large enzyme of 2,620 amino acids, crystallizing it may be difficult.

Nevertheless, a Cryo-EM structure might be possible.[65] On the other hand, machine learning and artificial intelligence-based platforms have made it possible to get near-native predicted models.[16]

Given the involvement of USP24 in various diseases, this dynamic DUB is becoming an attractive drug target, highlighting the importance of developing potent and selective USP24 inhibitors. Only two compounds inhibiting USP24 have been reported so far, both of which have not been thoroughly characterized. This clearly shows that there is still a long road ahead towards identifying better compounds. WP1130 is a pan-DUB inhibitor that targets many cysteine DUBs[66] and the perazine-derived compound NCI677397 is expected to bind many other protein targets, including DUBs, as has been shown for trifluoperazine, for example.[67] It is, therefore, unlikely that either of these compounds will emerge as specific USP24 inhibitors.

Based on the experience of targeting other DUBs, isolating the USP24 catalytic domain through recombinant production appears to be a feasible strategy.[68] This smaller domain (aa 1686-2047) resembles the USP7 catalytic domain, making it amenable for recombinant production compared to the full-length USP24 **(see Outstanding Questions)**. High-throughput platforms can be employed to screen large compound libraries or validate compounds in orthogonal assays. Setting up DUB activity monitoring assays based on fluorescence polarization or intensity readout using substrate-mimicking and DUB labeling probes can further help in investigating USP24's catalytic activity.[69]

Virtual screening can be a useful path to follow. This already yielded NCI677397 from such a screening effort, serving as a positive reference for future screens[11]. Structural homology and sequence alignment have identified similarities in USP24 and USP7 catalytic pockets, suggesting the possibility of repurposing potent USP7 inhibitors for USP24 inhibition or applying USP7 inhibitors as a starting point for future USP24 inhibitor design. Within the same line of reasoning, a similar strategy could be applied for USP9x, as being the closest relative of USP24 and for which inhibitors have been reported.[61, 70] **(see Outstanding Questions)**.

Finally, it is worth noting that unlike other DUBs, USP24 presents a multifaceted function and the undiscovered structural integrities offer an exciting opportunity for drug developers to target this enzyme. With well-planned approaches and the right assay tools, the full potential of USP24 as a therapeutic target can further enhance our understanding of DUBs in crucial cellular pathways.

Outstanding questions and challenges

1. What are the biological substrates of USP24: Ubiquitinated or ISGylated?
2. What kind of Ub linkages does USP24 prefer to cleave apart from K63?
3. Apart from the UBA and the catalytic domain, what is the function of the armadillo repeats, the rest big part of USP24? Are there domains with different functionality?
4. Can USP7 and USP9X inhibitors be repurposed for USP24?

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