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Review

Targeting UFMylation in Cancer: Emerging Roles in Cancer Stem Cell Regulation and Therapeutic Targeting

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Abstract

UFMylation is an emerging ubiquitin-like post-translational modification involved in protein quality control, endoplasmic reticulum (ER) homeostasis, and cellular responses to stress. Increasing evidence links UFMylation to cancer development and progression, revealing tumor-promoting and tumor-suppressive functions that depend on tumor type, cellular context, and the substrates involved. Recent discoveries also implicated UFMylation in cancer stem cell (CSC) maintenance, particularly through its effects on stemness-associated signaling pathways, transcription factors, tumor sphere formation, and therapeutic resistance. This review outlines the molecular mechanisms and regulation of UFMylation and examines its context-dependent functions in cancer, with particular emphasis on CSC biology. We discuss how alterations in the UFMylation pathway may influence stem-like properties and treatment responses. We also examine therapeutic strategies for targeting the UFMylation pathway, including pharmacological inhibitors, modulation of transcription factors, and combination approaches aimed at improving treatment responses. Particular attention is given to the potential of inducing ferroptosis through UFMylation inhibition as a strategy for targeting CSCs. Finally, this review highlights principal knowledge gaps and future research directions, emphasizing the need to characterize novel relevant substrates, validate observations in physiologically accurate settings, and implement in vivo tumor models to better understand the role of UFMylation in cancer stemness.

Keywords: ubiquitin-like modification; UFMylation; tumorigenesis; cancer stem cells; therapy

1. Ubiquitination and UFMylation: Similarities and Differences

Post-translational modifications (PTMs) regulate protein homeostasis and are involved in nearly all cellular processes, including DNA damage repair, signal transduction, cell proliferation, stress responses, and cell death [1–3]. Most cellular proteins undergo one or more PTMs during or after protein biosynthesis. By covalently adding or removing specific chemical groups from amino acid residues, PTMs can influence protein activity, stability, localization, and interactions with other proteins, thereby considerably expanding the functional information encoded by the genome [3]. Phosphorylation, ubiquitination, acetylation, methylation, glycosylation, and palmitoylation are among the best-characterized PTMs, along with non-enzymatic modifications such as glycation and nitrosylation.

Ubiquitination has a central role in regulating protein turnover and signaling. It is mediated by a sequential enzymatic cascade involving E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and E3 ubiquitin ligases. Ubiquitination generally occurs through a three-step, ATP-dependent process in which ubiquitin is first activated by an

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E1 enzyme, then conjugated to ubiquitin-carrier E2 enzymes, and finally ligated to lysine residues of target proteins via specificity-conferring E3 ubiquitin ligase complexes [4]. A target lysine site can be modified by mono-ubiquitination or by poly-ubiquitination, in which multiple ubiquitin molecules form a chain, with each additional ubiquitin added to a lysine residue of the previous one. Classical K48-linked polyubiquitination directs proteins to proteasomal degradation. As with other reversible PTMs, E3 ligases are counteracted by eraser enzymes, called deubiquitinases. In addition, numerous non-proteolytic regulatory roles of mono- and poly-ubiquitination with other linkage modes have been described [5]. Ubiquitination is rarely the primary point of regulation; it is often preceded by the addition or removal of other PTM signals at or near the ubiquitination site. These PTMs act as recognition sites for reader proteins that interpret and integrate upstream signals, providing an additional layer of rapid and reversible regulation and fine-tuning before irreversibly committing a target protein to degradation [6].

The ubiquitin system is complemented by a growing family of ubiquitin-like modifiers (UBLs). Although UBLs generally have limited sequence similarity to ubiquitin, they share a characteristic ubiquitin-fold structure and are conjugated to their targets through related enzymatic cascades involving E1-, E2-, and E3-like enzymes [7,8]. Unlike ubiquitination, which is classically associated with protein degradation, many UBLs mainly regulate protein function by affecting protein–protein interactions, subcellular localization, stability, or activity. These include small ubiquitin-like modifier (SUMO), neural precursor cell-expressed developmentally downregulated 8 (NEDD8), and interferon-stimulated gene 15 (ISG15). These modification systems are not completely independent, and crosstalk between ubiquitin and UBL pathways contributes to the fine regulation of cellular homeostasis, particularly under conditions of cellular stress [9].

UFM1 (ubiquitin-fold modifier 1) is another member of the UBL family and was identified in 2004 as a novel ubiquitin-like protein [10]. Its conjugation pathway, known as UFMylation, has received increasing attention over the past decade. Initially associated primarily with cellular stress responses and protein quality control at the endoplasmic reticulum (ER), a major site of its activity, UFMylation is now implicated in a broader range of cellular processes, including protein translation, DNA damage responses, autophagy, immune regulation, tumorigenesis, and cancer progression [11–13].

Similar to ubiquitination, UFMylation involves a three-step enzymatic cascade, although it has a more limited set of known components and substrates than other UBL systems. The UFMylation cycle begins with proteolytic maturation of precursor UFM1, primarily mediated by UFM1-specific proteases UFSP1 and UFSP2, which expose its C-terminal glycine for conjugation. Mature UFM1 is then activated by UBA5 in an ATP-dependent reaction and transferred to UFC1, followed by its conjugation to target proteins through the UFL1-containing E3 complex. The pathway is reversible, with UFSP1 and UFSP2 acting as de-UFMylases that remove UFM1 from modified substrates [11,12] .

2. Physiological Functions of UFMylation

One of the best-established functions of UFMylation is its role in ER-associated ribosome quality control. The synthesis of secretory and membrane proteins takes place at the ER, where translation and protein translocation must be tightly coordinated. When translation is interrupted, ribosomes can become stalled at the ER translocon, creating a situation that must be resolved to maintain both protein synthesis and ER homeostasis. The UFMylation machinery is localized to the cytosolic surface of the ER and is closely associated with ER-bound ribosomes [14,15]. Ribosomal protein 26 (RPL26) has emerged as the principal physiological target of UFMylation. Its modification is induced in response to ribosome stalling and is involved in the processing and recycling of stalled ribosomes at

the ER. More recent structural studies have provided further insight into how the UFM1 machinery recognizes the 60S ribosomal subunit and coordinates the release of stalled ribosomes from the ER translocon [11,16].

Beyond its role in ribosome-associated quality control (RQC), UFMylation contributes to broader ER homeostasis by regulating responses to ER stress and supporting the selective degradation of damaged ER components through ER-phagy [17]. For example, UFMylation of the ER-associated protein CYB5R3 has been linked to its lysosomal degradation through an ER-phagy pathway involving CDK5RAP3 [17]. Disruption of the UFMylation pathway has also been associated with impaired proteostasis and altered unfolded protein response signaling [18,19]. Together, these findings suggest that UFMylation may coordinate several ER quality-control mechanisms, allowing cells to respond to increased demands on protein synthesis and folding. This may be particularly important in cells with high secretory activity or under conditions in which protein homeostasis is challenged [20].

UFMylation also appears to have functions outside the ER. Components of the UFM1 system have been linked to DNA damage responses, inflammation, and immune regulation [13,20]. For example, UFL1 and DDRGK1 are recruited to sites of ribosomal DNA damage, where they contribute to ATM activation and regulation of rDNA transcription [20]. UFMylation has also been linked to replication fork processing through modification of PTIP, especially in BRCA1-deficient cells [21,22]. In addition, UFMylation of MRE11 following DNA double-strand breaks promotes assembly of the MRE11-RAD50-NBS1 complex and ATM activation, thereby facilitating the DNA damage response [23]. In the context of immune regulation, UFMylation has been linked particularly to the regulation of innate immunity and antiviral signaling. UFL1 supports RIG-I/MAVS-mediated signaling and downstream interferon production, whereas virus-induced UFMylation of MAVS can promote its lysosomal degradation and suppress inflammatory signaling [24,25].

Despite recent advances in UFMylation research, the identification of physiologically relevant substrates remains a key challenge in the field. Several proteins have been identified as UFM1 substrates, either through proteomic studies or in specific disease models. Some of these substrates have already been discussed above in the context of ER homeostasis, DNA damage response, and immune signaling, such as RPL26, CYB5R3, MRE11, PTIP, and MAVS [16,21,23,25,26]. Other reported substrates include HRD1, p53, and ribosomal protein 10 (RPL10), further illustrating the diverse cellular processes regulated by UFMylation [27–29]. However, detecting UFMylation under experimental conditions does not necessarily mean that a protein is a physiological substrate. RPL26 currently has some of the strongest evidence as a physiological UFM1 substrate, while other reported substrates may be more context-dependent [14,26]. Recent advances in UFMylome profiling have substantially improved substrate identification by combining optimized approaches that enhance detection of low-abundance UFMylation events with site-specific peptide enrichment and mass spectrometry strategies that enable their analysis under in vivo conditions [30,31]. These approaches provide valuable tools to distinguish physiologically relevant UFMylation targets from experimentally detected modifications and to attribute cellular phenotypes caused by UFMylation disruption to modification of a specific substrate. Nevertheless, further biochemical and genetic studies are needed to better define the physiological UFMylation targets and understand how substrate selection is regulated.

3. The Role of UFMylation in Cancer Biology

Growing interest in UFMylation's role in cancer stems directly from its fundamental importance in maintaining cellular homeostasis. Because tumor cells constantly face proteotoxic, metabolic, oxidative, and genotoxic stress, they rely heavily on specialized mechanisms to adapt to such harsh conditions. Given its role in protein quality control, ER

homeostasis, and cellular stress responses, UFMylation may influence the ability of tumor cells to survive and adapt to the stressful tumor microenvironment [12,16,32,33]. UFMylation can therefore be considered a multimodal regulatory hub that integrates adaptive mechanisms with metabolic regulation, DNA damage responses, and immune signaling, with potential implications for tumor growth and stem cell plasticity. Within this framework, the biological effects of UBA5, UFC1, and UFL1 depend on the substrates modified and the signaling pathways engaged. Because altered expression of UFMylation components alone does not prove changes in pathway activity or explain their impact on tumor behavior, expression levels must be interpreted alongside evidence of substrate UFMylation and its functional consequences.

Several studies have linked UFMylation to different aspects of cancer biology, including tumor-cell proliferation, survival, stress adaptation, and immune regulation. One of the earliest direct connections between UFMylation and cancer was described in breast cancer, where estrogen stimulation induced UFMylation of the transcriptional coactivator ASC1. Estrogen-bound ERα displaced UFSP2 from ASC1, allowing its UFMylation and promoting the recruitment of transcriptional coactivators to ERα target genes, while disruption of the UFMylation machinery or mutation of the UFMylation sites in ASC1 impaired tumor growth [34]. This study provided early evidence connecting hormonal signaling to reversible modification of a transcriptional regulator, illustrating how UFMylation can translate an extracellular signal into a tumor-promoting transcriptional response. UFMylation has subsequently been implicated in several other aspects of tumor biology. For instance, in pancreatic adenocarcinoma, UFMylation of RPL10 was associated with increased tumor-cell proliferation and stemness through regulation of the transcription factor Klf4 [29]. Beyond its effects on tumor growth and stemness, UFMylation has also been linked to cancer cell migration, invasion, and metastasis. A study by Zhao et al. showed that Akt-mediated phosphorylation of UFL1 promotes these processes by enhancing UFMylation of ArpC4, a core subunit of the Arp2/3 complex involved in lamellipodia formation and invasive tumor behavior [35]. Here, UFL1 provides a functional link between Akt signaling and the cytoskeletal machinery controlling cell movement. This example also shows how phosphorylation can regulate substrate engagement and the consequences of UFMylation, beyond changes in UFL1 protein levels.

Although UFMylation has been linked to tumor growth and progression across several cancer types, its effects appear to depend on the tumor type, cellular environment, and specific proteins affected [33]. For example, UFMylation supports ERα-mediated transcriptional activity in breast cancer cells [34] and has also been implicated in regulating cancer stemness, proliferation, and antitumor immune response in other cancer models [29,34,36,37]. However, recent studies have also identified tumor-suppressive functions of UFMylation. In colorectal cancer, UFL1 suppresses tumor progression and glycolysis by UFMylating and inhibiting the enzyme ENO1. This modification disrupts ENO1 dimerization and reduces its enzymatic activity, directly linking substrate UFMylation to glycolytic flux regulation. Furthermore, pharmacological enhancement of the UFL1–ENO1 axis using the antibiotic torezolid significantly boosts the antitumor effectiveness of chemotherapy in preclinical models [38]. These findings illustrate how specific UFL1–substrate interactions can suppress tumor metabolism and influence treatment response. Similarly, UFMylation of PHLDA3 inhibits Akt signaling and tumor growth in lung adenocarcinoma [39], whereas loss of UFL1 drives chromosome instability and tumorigenesis of prostate cancer [40]. In hepatocellular cancer, UFMylation suppresses metastasis by inhibiting β-catenin-driven hybrid EMT and NK cell evasion [41]. Consistent with these tumor-suppressive effects, Liang and colleagues demonstrated that UFMylation stabilizes KEAP1, a negative regulator of NRF2, linking UFMylation to redox regulation through the control of KEAP stability.

Specifically, UFL1 deficiency in hepatocytes promoted KEAP1 degradation and subsequent NRF2 activation, thereby promoting liver tumorigenesis [42]. Together, these findings suggest that UFMylation does not have a single role in cancer but may exert different effects depending on where and under which conditions the pathway is altered. Clarifying the role of UFMylation in different tumor types requires a better understanding of these variations, particularly regarding specific substrates and underlying cellular stress conditions.

Another emerging area is the association between UFMylation and tumor–immune system interactions, particularly through the regulation of immune-checkpoint receptors. In tumor cells, UFMylation of PD-L1 promotes its ubiquitination and degradation, whereas disruption of UFL1 or UFM1 increases PD-L1 stability and can impair antitumor immunity [37]. Conversely, in triple-negative breast cancer, UFMylation indirectly reduces PD-L1 ubiquitination and degradation by stabilizing PLAC8, thereby promoting tumor cell proliferation and immune evasion [43]. Similarly, in T cells, UFL1-dependent stabilization of PD-1 inhibits its ubiquitination and degradation, while UFL1 loss reduces PD-1 stability and enhances antitumor T-cell activity. The same study showed that AMPK phosphorylates UFL1, reducing PD-1 UFMylation and promoting its degradation [36]. This positions UFL1 at the intersection of AMPK signaling and immune-checkpoint regulation, illustrating how upstream pathways modulate substrate UFMylation to influence immune cell function.

These findings indicate that the consequences of altering UFMylation may depend strongly on the cellular compartment in which the pathway is affected. Across the examples discussed above, UFMylation is both regulated by upstream signals and directly involved in modifying downstream processes, including transcription, cytoskeletal remodeling, metabolism, and immune-checkpoint stability. Together with its functions in protein homeostasis, metabolic regulation, and genomic stability, these findings support a broader conceptualization of UFMylation as a regulator that shapes how tumor and immune cells adapt to changing conditions. However, many of these cancer-associated functions were identified relatively recently, and it remains unclear whether these mechanisms act together within a given tumor, which of them become functionally dominant under particular stress conditions, and whether they reflect shared or tumor-specific functions of UFMylation across different tumor types. In particular, the functional relevance of individual UFMylation substrates and the circumstances in which tumor cells become dependent on this pathway remain to be fully established.

4. UFMylation and Cancer Stem Cells

Although UFMylation has been implicated in various aspects of cancer biology, its role in cancer stemness remains underexplored. Its potential relevance can be considered through two complementary functions: regulating stemness-associated programs and supporting cellular processes that enable stem-like cells to adapt to stress and survive. Importantly, functional findings obtained in CSC models should be distinguished from associations in tumor tissues and mechanisms established in other cellular systems.

One of the clearest substrate-specific examples comes from pancreatic adenocarcinoma, where Wang et al. identified RPL10 as a direct UFMylation substrate in pancreatic cancer tissues and cell lines and linked this modification to increased Klf4 expression, proliferation, and stemness-associated features. Furthermore, reducing RPL10 UFMylation, either by UFL1 knockdown or mutation of the identified UFMylation sites, decreased sphere formation and stemness-associated features [29]. These findings suggest that modification of a specific UFMylation substrate supports the maintenance of stem-like properties by indirectly regulating Klf4 expression, although they do not establish whether this mechanism operates in other cancer types.

Stem-like tumor cells need to maintain protein homeostasis while adapting to hypoxia, nutrient limitation, oxidative stress, and other unfavorable conditions. Because of its involvement in proteostasis and adaptive stress response, UFMylation may play an important role in helping these cells maintain homeostasis under such conditions. The potential roles of UFMylation in cancer stem cells are as follows: (1) maintaining stemness and self-renewal, (2) mediating chemoresistance and survival, and (3) crosstalk with key oncogenic pathways. In addition to the previously described role of RPL10 UFMylation in pancreatic cancer stemness [29], our studies provide further functional evidence linking the UFMylation pathway to CSC-associated features in head and neck squamous cell carcinoma (HNSCC) cells. UFM1 silencing reduced sphere number and size and decreased stemness-associated markers, supporting the UFMylation pathway's contribution to the maintenance of stem-like properties. We also identified Sp1 as a potential transcriptional regulator of the UFMylation pathway and demonstrated that its inhibitor mithramycin markedly decreased CSC sphere formation, survival, and stemness-associated markers, along with decreases in several UFMylation components and UFM1 conjugate levels [44]. However, because mithramycin affects transcription more broadly, these effects cannot be attributed exclusively to UFMylation inhibition. Together, the genetic and pharmacological findings support pathway involvement, while relevant substrates and downstream mechanisms remain to be identified.

Evidence supporting the importance of UFMylation in stem-like cancer cells also comes from glioblastoma, where genetic disruption of key UFMylation pathway components impaired the growth and survival of patient-derived glioblastoma stem cells [45]. These findings show that the pathway is important in a CSC model but do not, by themselves, distinguish whether it helps maintain stem-like properties or supports cell growth and survival more generally. Similarly, our finding that altered expression of UFMylation components, particularly UFSP2, in HNSCC tumor tissues was associated with increased stemness-related markers and aggressive clinicopathological features provides clinical support for a link between UFMylation and cancer stemness, complementing functional evidence obtained in CSC models [46]. Sphere formation and stemness-associated markers provide valuable evidence of stem-like properties, especially when interpreted alongside cell viability and proliferation, while complementary assays can further clarify their relevance to long-term self-renewal and tumor-initiating capacity.

UFMylation may connect stemness-associated signaling with stress adaptation through effects on different substrates. For example, YAP has recently been identified as a UFMylation substrate whose modification enhances its stability and transcriptional activity in kidney-development models [47]. Given the established role of YAP as a master transcriptional coactivator and central effector of the Hippo signaling pathway involved in CSC maintenance and cellular plasticity, its regulation by UFMylation highlights a mechanism worth investigating in the context of CSCs and cancer stemness.

Concerning therapy resistance, UFMylation may help CSCs survive standard treatments such as radiation and chemotherapy by supporting protein homeostasis and adaptive stress responses. Dysregulation of UFMylation components is associated with cancer stemness, epithelial–mesenchymal transition (EMT), and ferroptosis resistance in certain tumor models, pointing to a potential role in stress adaptation and therapy sensitivity. We previously demonstrated that elevated levels of UFM1 directly correlate with poor overall survival and treatment failure in HNSCC [44]. UFMylation may protect CSCs from proteotoxic stress and oxidative stress-induced cell death, potentially through mechanisms such as SLC7A11 stabilization and suppression of ferroptosis. SLC7A11 is a key component of the cystine/glutamate antiporter system Xc-, which imports cystine for glutathione (GSH) synthesis. Studies show that UFMylation directly stabilizes SLC7A11 and prevents its degra-

dation, thereby maintaining high SLC7A11 and GSH levels and supporting GPX4-mediated antioxidant defense. Through this mechanism, UFMylation helps limit iron-dependent lipid peroxidation and suppresses ferroptosis. Consistent with these findings, we recently showed that pharmacological inhibition of UBA5 reduces SLC7A11 and GPX4 mRNA expression and induces ferroptosis-associated cell death in HNSCC tumor spheres, suggesting that targeting UFMylation may sensitize CSCs to ferroptosis-inducing therapies [46,48,49].

UFMylation may also support stem-like cancer cells by maintaining the cellular conditions required for survival. Its established role in endoplasmic reticulum-associated degradation (ERAD) and RQC provides a mechanistic basis for investigating how CSCs tolerate proteotoxic and treatment-induced stress [20,27], processes that may help CSCs maintain protein homeostasis despite their high metabolic requirements and exposure to environmental or therapy-induced stress. However, the contributions of specific quality control mechanisms to CSC survival and treatment resistance must be validated directly in CSCs, rather than assuming their roles based on other cell models.

Components of the UFMylation system (e.g., via CDK5RAP3 and UFL1 complex interactions) have also been implicated in key oncogenic pathways, including Wnt/β-catenin and NF-κB signaling, which regulate processes critical for CSC maintenance, influencing cell survival, migration, and invasion depending on the tumor type [50]. These connections suggest additional mechanisms through which the UFMylation system may influence stem-like properties. However, effects involving individual pathway components should be distinguished from those demonstrated to require covalent substrate UFMylation. It is also crucial to emphasize the dual, context-dependent role of UFMylation in cancer: while it stabilizes key tumor suppressors such as p53 by preventing their proteasomal degradation [28], it can also stabilize oncogenic drivers to support tumor-initiating and tumor-promoting capacities [34,51,52]. In CSC microenvironments, where p53 mutations are common and protein synthesis and folding demands increase, along with severe microenvironmental stress (hypoxia, nutrient deprivation), UFMylation may regulate substrates that support stemness and survival. Rather than enforcing p53-driven apoptosis, UFMylation might stabilize pro-survival and oncogenic drivers. This has been demonstrated for ASC1/ERα, HIF-1α, PARP1, SLC7A11, Pirin, and PLAC8 [34,43,51,53]. In particular, its effects on HIF-1α and PARP1 connect UFMylation with hypoxic adaptation and genome maintenance, respectively. By promoting protein stability and cellular adaptation to stress, these UFMylation-mediated mechanisms may complement pathways associated with CSC maintenance and tumor progression by supporting survival under stress. However, their specific contribution to CSC biology and treatment resistance remains to be explored.

Together, these findings support a framework in which UFMylation contributes to cancer stemness through substrate-specific regulation of stem-like properties and broader support of cellular stress tolerance. This combination of functions highlights its potential multimodal role in CSC biology. Whether UFMylation also regulates transitions between stem-like and differentiated states remains an open question. Further studies combining substrate-specific perturbation and rescue experiments with assessments of self-renewal and tumor initiation will help clarify which effects reflect regulation of stem-like properties and which arise from broader changes in cell growth and survival.

5. Targeting the UFMylation Pathway in Cancer

Given these diverse functions, UFMylation has also attracted increasing interest as a potential therapeutic target. Inhibition of core components such as UBA5, UFC1, or UFL1 can disrupt the ability of tumor cells to maintain proteostasis and adapt to cellular stress, whereas modulating the de-UFMylating enzymes, particularly UFSP2, offers another promising therapeutic strategy [33]. However, therapeutically targeting this pathway may

be challenging given the critical role of UFMylation in normal cellular homeostasis. A better understanding of UFMylation substrates and the conditions under which cancer cells become dependent on this pathway is therefore essential for determining the cellular contexts in which this pathway can be selectively targeted.

UFMylation is a multi-step cascade (E1: UBA5, E2: UFC1, E3: UFL1, and deUFMylases: UFSP1/2), and drug discovery efforts focus on several approaches: (1) inhibiting key enzymes, (2) targeting upstream transcriptional regulators, and (3) exploring therapeutic combination strategies (Table 1). Among these, direct small-molecule inhibitors of UFMylation enzymes have received particular attention (Figure 1).

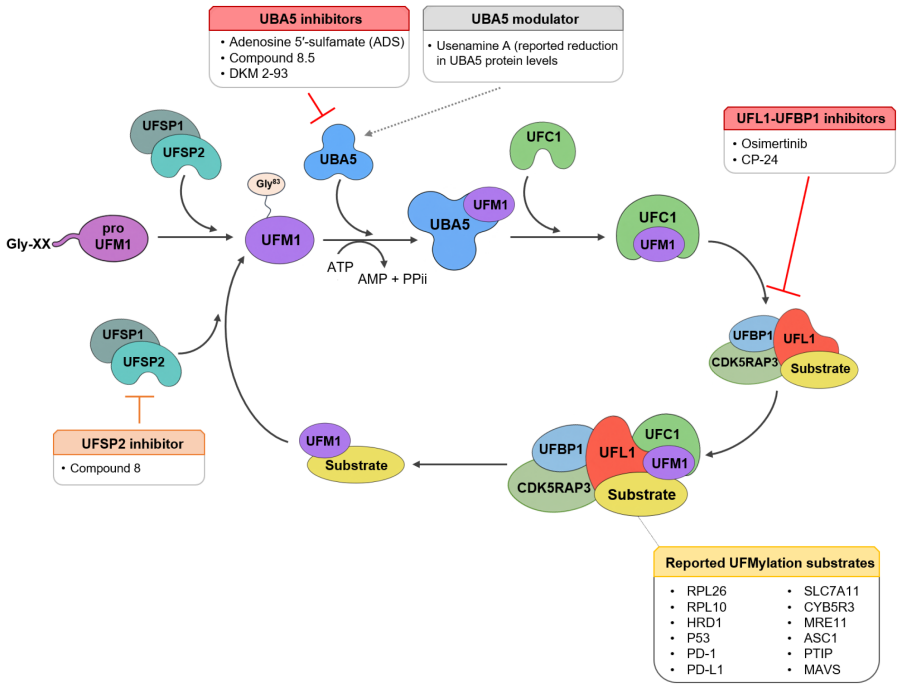


Figure 1. Schematic representation of the UFMylation pathway, representative substrates, and pharmacological modulators. The UFMylation pathway involves a series of enzymatic reactions that mediate the reversible conjugation of UFM1 to target proteins. The enzymatic cascade is initiated by proteolytic maturation of precursor UFM1 by UFSP1 and UFSP2 to expose its C-terminal glycine residue (Gly83). Mature UFM1 is then activated by UBA5 in an ATP-dependent reaction and subsequently transferred to the conjugating enzyme UFC1. UFM1 is then conjugated to target substrates through the UFL1-UFBP1 (DDRGK1) ligase complex, with CDK5RAP3 acting as an associated regulatory factor. UFSP1 and UFSP2 also catalyze the de-UFMylation step, releasing UFM1 for subsequent conjugation cycles. We indicate selected reported UFMylation substrates involved in proteostasis, DNA damage responses, and immune signaling. Emerging pharmacological modulators targeting components of the UFMylation machinery are distinguished by color, with blunt-ended lines indicating inhibition of the specified component or interaction. Unlike compounds that inhibit UFM1 conjugation (red), Compound-8 inhibits UFSP2-mediated de-UFMylation (orange) and was reported to increase UFM1-conjugate levels. UBA5 modulators are grouped by their reported effects. The solid red blunt-ended line from the UBA5 inhibitor box indicates direct inhibition of UBA5 enzymatic activity. The gray dotted arrow from the UBA5 modulator box indicates the reported reduction in UBA5 protein levels following usenamine A treatment.

5.1. Modulation of Core UFMylation Enzymes

Among the UFMylation pathway components, UBA5 has emerged as a promising target for direct pharmacological inhibition. Selective small-molecule UBA5 inhibitors can interfere with its activity by competing with ATP or blocking UFM1-UBA5 thioester bond formation [54]. These inhibitors may disrupt ER homeostasis and activate ER stress

responses in cancer cells [55], while also reducing global UFMylation and HIF1α stability in hypoxic tumor niches, ultimately suppressing tumor growth in preclinical models [51].

Although UBA5 plays an important role in different human diseases [31,56,57], relatively few UBA5 inhibitors have been discovered. Among the best-characterized compounds are adenosine 5′-sulfamate (ADS), Compound 8.5, and DKM 2-93, while the natural product Usenamine A has been reported to reduce UBA5 protein levels. ADS is an ATP-competitive analogue with an IC50 of 13 μM, but its utility is limited because it inhibits many E1 enzymes, not just UBA5. Compound 8.5 is a selective UBA5 inhibitor with an IC50 of 4 μM, though its status as a metal chelator (binding to zinc) can lower its potential as a drug. DKM 2-93 is a covalent UBA5 inhibitor that works through a different mechanism and has relatively low potency, with an IC50 of 430 μM. In our recent study, we found that lower concentrations of DKM 2-93 increased the viability of Detroit 562 HNSCC tumor spheres, alongside increased stemness marker expression and an autophagy-related response reflected by an increase in LC3B-I/II levels. However, higher concentrations reduced sphere viability, with evidence of ferroptosis-associated cell death [46]. The low-dose response may reflect an adaptive response in stem-like cells. Therefore, evaluating DKM 2-93's potential to target CSCs will require assessing both sphere viability and long-term self-renewal across a range of concentrations. The same inhibitor reduced proliferation of patient-derived glioblastoma stem cells [45]. Usenamine A is a natural product that reduces UBA5 expression levels in breast cancer cells, subsequently decreasing UFMylation and inducing ER stress, apoptosis, and autophagy. However, it remains unclear whether usenamine A interacts directly with the UBA5 protein or inhibits its enzymatic activity, suggesting that its effects may indirectly affect the UFMylation pathway [55].

More recently, Singh et al. identified five small-molecule UBA5 inhibitors spanning three chemical scaffolds, with low-micromolar activity in biochemical assays. Inhibition was confirmed using complementary assays measuring UBA5-mediated UFM1 activation, and selected compounds effectively suppressed UFMylation. Under the tested conditions, these compounds exhibited preferential inhibition of UBA5 over UBA1 without significantly affecting global cellular polyubiquitination. They provide additional chemical tools for investigating UFMylation, although further optimization and preclinical validation are required [54]. Combining pharmacological studies with genetic manipulation and rescue experiments would help distinguish effects caused by UFMylation inhibition from other cellular effects of the compounds. Measuring substrate UFMylation could further clarify how target inhibition leads to the observed cellular responses.

As an enzyme involved in both UFMylation and de-UFMylation, UFSP2 represents another potential target for pharmacological modulation of the UFMylation pathway. Although UFSP2 contributes to UFM1 precursor maturation, it also removes UFM1 from modified substrates. UFSP1 can support UFM1 maturation in the absence of UFSP2, helping to explain why loss of UFSP2 can increase substrate UFMylation [58]. Inhibition of UFSP2-mediated de-UFMylation therefore represents a distinct strategy from blocking UFM1 conjugation. Compound-8, a covalent UFSP2 inhibitor, dose-dependently enhanced the global UFMylation while decreasing PD-L1 expression. These findings indicate enhanced accumulation of UFMylated proteins rather than inhibition of UFM1 conjugation. The authors also observed the tumor-suppressive effect of Compound-8 in a mouse tumor model. In a mouse model, the authors confirmed the tumor-suppressive activity of Compound-8. While monotherapy with either Compound-8 or PD-1 blockade significantly delayed tumor growth, their combination yielded superior antitumor efficacy without causing noticeable toxicity or weight loss [37].

Although no direct UFL1 inhibitor is currently available, Tan and colleagues recently identified two compounds targeting DDRGK1, a core protein in the UFMylation E3 ligase

complex: osimertinib (a drug originally made as an EGFR T790M inhibitor that now shows a new covalent mechanism) and CP-24 (a brand-new non-covalent inhibitor). Both compounds disrupt the DDRGK1-UFL1 interaction, reducing global UFMylation, inhibiting ER-phagy, and inducing ER stress [59]. Unlike conventional catalytic E3 ligases, UFL1 lacks a catalytic cysteine and relies on scaffold proteins like DDRGK1 (UFBP1) and CDK5RAP3 to target substrates. Therefore, disrupting interactions within this complex, particularly the UFL1-DDRGK1 interaction, using small molecules or peptide mimetics represents an alternative strategy for preventing substrate UFMylation without directly inhibiting a catalytic active site.

5.2. Targeting Transcriptional Regulation of UFMylation

In addition to directly inhibiting components of the UFMylation system, modulation of upstream regulators controlling their expression may provide an alternative strategy for interfering with the pathway. We previously identified Sp1 as the primary transcriptional regulator of several UFMylation pathway genes, demonstrating that its inhibition by mithramycin downregulates UFMylation gene and protein expression in HNSCC cells. In tumor spheres, mithramycin treatment similarly triggered apoptosis and suppressed EMT-related features [44]. However, because mithramycin causes severe toxicity across multiple organ systems, developing mithramycin analogs (mithralogs) with better safety and pharmacological profiles is a promising alternative.

Beyond transcriptional regulation of UFMylation pathway genes, UFMylation has also been linked to the regulation of stemness-associated transcription factors, including Klf4, whose expression and downstream transcriptional activity are enhanced by RPL10 UFMylation [29]. Together, these findings underscore the close relationship between UFMylation and transcriptional regulation, suggesting that interfering with these interactions may offer additional opportunities to target cancer stemness.

Alongside transcriptional regulation, certain drugs and targeted delivery systems have been reported to modulate UFMylation without directly inhibiting its core enzymes. For instance, in breast cancer models, metformin suppressed UFMylation of SLC7A11, thereby reducing its stability and promoting ferroptosis [49]. In pancreatic cancer models, lipid nanoparticles delivering siRNA targeting macrophage-capping protein (MCP) reduced Pirin UFMylation and GPX4 transcription, promoting ferroptosis and antitumor immune response [53]. Although these insights offer novel therapeutic options for targeting the UFMylation pathway, their precise contribution to overall treatment response must still be defined.

5.3. Combination Strategies for Targeting UFMylation

Targeting UFMylation may enhance the efficacy of existing antitumor therapies. One promising strategy includes combination with immune checkpoint blockade, such as anti-PD-1/anti-PD-L1 therapy. Because UFMylation stabilizes HIF1α and drives an immunosuppressive microenvironment in hypoxic tumors, pharmacologically inhibiting UBA5 promotes HIF1α degradation, attenuates immunosuppressive signaling, and sensitizes tumors to anti-PD-1 therapy in preclinical models [51]. In addition, targeting UFMylation may also help overcome chemotherapy resistance. Because UFMylation aids in DNA repair and mitigates ER stress from DNA-damaging agents, pairing UBA5 or UFL1 inhibitors with agents like cisplatin, doxorubicin, or 5-FU restores chemosensitivity in aggressive tumors (e.g., breast, gastric, and glioblastoma) [57].

Table 1. Compounds targeting the UFMylation pathway.

Target Strategy	Compound	Mechanism of Action	Biological Effect	Reference
Upstream Transcription Inhibition	Mithramycin A/Sp1 Inhibitors	Suppresses Sp1-driven transcription of UFMylation machinery (UFM1, UBA5, UFSP2, DDRGK1, UFC1)	Depletes UFMylation levels, impairs tumor sphere formation, reduces EMT/stemness markers	[44]
UFMylation enzyme inhibitors	UBA5 inhibitors:			[37,46,54,55,59,60]
	ADS	By physically locking UBA5 in an inactive, adduct-bound state, ADS prevents subsequent cycles of ATP binding, UFM1 activation, and transfer to UFC1	Inhibiting UBA5 via ADS disrupts key processes maintained by UFMylation, particularly endoplasmic reticulum (ER) stress management, ribosome-associated protein quality control (RQC), and DNA double-strand break repair.	
	Compound 8.5 (UBA5-IN-1)	Noncompetitive inhibitor; acts via a zinc-chelating mechanism, binding to structural elements or allosteric sites on UBA5 to disrupt its structural integrity or catalytic efficiency.	Prevents the UFMylation of specific downstream regulatory proteins—such as HIF1α altering their stability, stress-response signaling, and turnover, and enhances the efficacy of combination treatments, such as anti-PD-1 immunotherapies.	
	DKM 2-93	DKM 2-93 covalently binds to the active-site catalytic cysteine residue (Cys250) of UBA5 by irreversibly reacting with Cys250; DKM 2-93 prevents UBA5 from forming the essential UBA5-UFM1 thioester intermediate.	Low doses cause autophagy; higher doses induce ferroptosis; apoptosis induction, ER stress, reduced tumor cell proliferation	
	Usenamine A	Reduces UBA5 protein expression in breast cancer cells. Direct inhibition of UBA5 catalytic activity has not been established.	Usenamine A disrupts endoplasmic reticulum (ER) proteostasis, triggering unresolved ER stress, inducing autophagy, and promoting apoptosis; suppresses cancer cell proliferation in breast cancer cells; anti-inflammatory potential	
	DDRGK1 inhibitors:			
	Osimertinib	Off-target small-molecule inhibitor: Although engineered to target EGFR's ATP-binding pocket (forming a covalent bond with Cys797), Osimertinib also disrupts the DDRGK1–UFL1 interaction by binding directly to DDRGK1, forming a covalent adduct.	Decreases UFMylation; UFMylation inhibition via Osimertinib promotes macrophage polarization toward an antitumor M1 phenotype; exhibits brain-penetrant pharmacokinetic properties and thus has potential for CNS malignancy treatment; antitumor effects in vitro and in vivo	
	CP-24	Non-covalent inhibitor that disrupts the physical interaction between DDRGK1 and UFL1	Downregulation/suppression of cellular UFMylation; impairs ER-phagy and triggers severe endoplasmic reticulum (ER) stress; suppresses tumor cell survival and reprograms the tumor immune microenvironment	
	UFSP2 inhibitor			
	Compound 8	Covalent Inhibitor of UFSP2; targets and forms a covalent bond with the catalytic cysteine residue (Cys294); blocks the ability to perform de-UFMylation	Accumulation and global enhancement of cellular UFMylation; contributes to immunotherapy with anti-PD-1	

Table 1. *Cont.*

Target Strategy	Compound	Mechanism of Action	Biological Effect	Reference
Therapeutic combination strategies	Combination with immune checkpoint blockade therapy (anti-PD-1/anti-PD-L1)	Inhibition of HIF-1α stabilization and modulation of PD-L1 stability by synergizing with its ubiquitination	Enhances antitumor efficacy of anti-PD1 therapy, reduces tumor size	[37,57]
	UBA5 inhibition + cisplatin	UBA5 inhibition enhances cisplatin sensitivity	UBA5 inhibition impedes cancer cell proliferation and cisplatin resistance	

While UFMylation research is still evolving, pharmacological targeting represents an important tool for strengthening its translational relevance. Emerging small-molecule inhibitors enable the investigation of context-dependent cancer vulnerabilities, treatment responses, and combinations with existing therapies, helping identify opportunities for targeted interventions. The effectiveness of these approaches may also depend on the metabolic environment. A recent preprint by Kunzmann et al. linked UFMylation dependence to extracellular alanine availability, highlighting how nutrient conditions shape the connection between protein quality control and metabolic adaptation [61]. Although the implications for drug sensitivity remain unclear, these findings suggest that nutrient availability should be considered when evaluating UFMylation-targeted interventions, both alone and in combination with other treatments. These results also show that while UFMylation primarily acts on ER-associated ribosomes, it coordinates a multi-organelle proteostasis network. The specific targets of this system and its broader impact on cellular fitness are governed by intrinsic cellular factors and nutrient availability.

6. Conclusions

Current evidence indicates that UFMylation plays diverse and context-dependent roles in cancer development and progression, with its effects varying according to tumor type, cellular context, and the specific substrates involved. These findings support a broader view of UFMylation as a multimodal regulatory hub integrating protein homeostasis and cellular stress responses with metabolism, genome maintenance, and immune signaling. Recent studies have also suggested that UFMylation may contribute to cancer stem cell (CSC) maintenance, particularly by regulating tumor sphere formation, enhancing stemness-associated markers, and interacting with key transcription factors. However, the mechanisms underlying these effects remain incompletely understood. Further advances in elucidating the molecular mechanisms and physiological functions of UFMylation, together with identification and functional validation of additional substrates, will be essential to clarify its contribution to cancer stemness. Future research should also prioritize in vivo tumor models and genetic gain- and loss-of-function experiments to establish the functional role of UFMylation in CSC maintenance, survival, and therapy resistance. Special focus is placed on the possibility of triggering ferroptosis by inhibiting UFMylation as an approach to target CSCs.

Given its involvement in critical oncogenic processes, UFMylation has emerged as an attractive target for therapeutic intervention. However, the importance of UFMylation in normal cellular homeostasis raises concerns about potential side effects, while the limited number and selectivity of currently available pharmacological inhibitors further hinder its therapeutic development. A better understanding of the conditions under which CSCs and other tumor cells become dependent on UFMylation, as well as the substrate-specific

mechanisms underlying its effects, will greatly facilitate the identification of cancer contexts in which this pathway can be effectively and selectively targeted.

By bringing together current knowledge of UFMylation mechanisms, physiological functions, cancer-associated roles, and its potential as a therapeutic target, this review highlights UFMylation as a promising area of research in CSC biology. A better understanding of the mechanisms linking UFMylation to cancer stemness will be crucial for determining whether this pathway can be selectively and effectively targeted in cancer therapy.

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Abbreviations

The following abbreviations are used in this manuscript:

ADS	Adenosine 5′-sulfamate
Akt	Protein Kinase B
ArpC4	Actin-Related Protein 2/3 Complex Subunit 4
ASC1	Activating Signal Cointegrator 1
ATM	Ataxia–Telangiectasia Mutated
BRCA1	Breast Cancer Type 1 Susceptibility Protein
CDK5RAP3	Cyclin-Dependent Kinase 5 Regulatory Subunit-Associated Protein 3
CSCs	Cancer Stem Cells
CYB5R3	Cytochrome b5 Reductase 3
DDRGK1	DDRGK Domain-Containing Protein 1
EMT	Epithelial–Mesenchymal Transition
ENO1	Enolase 1
ER	Endoplasmic Reticulum
ERAD	Endoplasmic Reticulum-Associated Degradation
ERα	Estrogen Receptor Alpha
GPX4	Glutathione Peroxidase 4
GSH	Glutathione
HIF-1α	Hypoxia-Inducible Factor 1-Alpha
HNSCC	Head and Neck Squamous Cell Carcinoma
HRD1	HMG-CoA Reductase Degradation Protein
ISG15	Interferon-Stimulated Gene 15
KEAP1	Kelch-Like ECH-Associated Protein 1
Klf4	Krüppel-Like Factor 4
MAVS	Mitochondrial Antiviral-Signaling Protein
MRE1	Metal Response Element 1
MRE11	Meiotic Recombination 11 Homolog
NBS1	Nijmegen Breakage Syndrome 1
NEDD8	Neural Precursor Cell-Expressed Developmentally Downregulated 8
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NK	Natural Killer
NRF2	Nuclear Factor Erythroid 2-Related Factor 2
PARP1	Poly [ADP-Ribose] Polymerase 1

PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
PHLDA3	Pleckstrin Homology-Like Domain Family A Member 3
PLAC8	Placenta-Specific 8
PTIP	PAX2 Transactivation Domain-Interacting Protein
PTMs	Post-Translational Modifications
RAD50	DNA repair protein RAD50
RIG-I	Retinoic Acid-Inducible Gene I
RPL10	Ribosomal Protein 10
RPL26	Ribosomal Protein 26
RQC	Ribosome-Associated Quality Control
SLC7A11	Solute Carrier Family 7 Member 11
Sp1	Specificity Protein 1
SUMO	Small Ubiquitin-Like Modifier
UBA5	Ubiquitin-Like Modifier-Activating Enzyme 5
UBLs	Ubiquitin-like Modifiers
UFC1	UFM1-Conjugating Enzyme 1
UFL1	UFM1-Specific Ligase 1
UFM1	Ubiquitin-Fold Modifier 1
UFSP1/2	UFM1-Specific Peptidase 1/2
YAP	Yes-Associated Protein

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