Proceedings of ICMMGH 2025 Workshop: Computational Modelling in Biology and Medicine
DOI: 10.54254/2753-8818/90/2025.GU20971

Advances in the Development of Ubiquitination Pathway Targets for the Treatment of Tumours

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Abstract: In recent years, oncology research has made great progress, but malignant tumours still become a great threat to human health, and many cancer treatments as well as targeted drug therapy for cancer have been developed, which can largely inhibit the development of cancer cells and even cure cancer. The research related to ubiquitination in cancer therapy research is also increasing year by year. Ubiquitination plays a very important role in protein localisation, metabolism, function, regulation and degradation. This also means that ubiquitination has a very important role in the treatment of tumour-related targets. This paper analyses the control of the ubiquitination pathway through the use of specific small molecule inhibitors. Such small molecule inhibitors inhibit a range of deubiquitinating enzymes so that negatively regulated proteins in the target molecule can be normally ubiquitinated and degraded, and t-cells can normally recognise and suppress tumourigenesis. Research into the effects of small molecule inhibitors on the ubiquitination pathway is making great progress today, which will be of great help in the future treatment of tumours. At the same time, the synergistic delivery of this approach with existing targeted drugs is also an important direction for future development that needs to be investigated.

Keywords: Deubiquitinating, Ubiquitin-Specific Protease (USPs), ML364, P5091.

1. Introduction

Despite significant advances in oncology research, cancer remains one of the world's most daunting health challenges. The growth and spread of malignant tumour cells remain a major threat to human health. Over the past few decades, significant progress has been made in the development of targeted therapies and drugs aimed at inhibiting tumour progression or even curing cancer [1]. These therapies typically focus on disrupting key molecular pathways that drive cancer development, such as signalling cascades, cell cycle regulation and apoptosis. However, despite these advances, cancer treatment remains a complex and elusive task due to the genetic heterogeneity of tumours, the development of drug resistance and the challenges posed by the tumour microenvironment (TMEs). Therefore, there is an urgent need for innovative cancer therapies [1,2].

The role of ubiquitination in cancer biology is one of the approaches that is receiving increasing attention. Ubiquitination is a post-translational modification that regulates the stability, localisation and function of proteins in cells. The process involves the attachment of small ubiquitin molecules to target proteins, marking them for degradation by the proteasome, or affecting their cellular function through other mechanisms. Ubiquitination is critical in maintaining intracellular homeostasis and

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DOI: 10.54254/2753-8818/90/2025.GU20971

regulating a variety of cellular processes, including cell cycle progression, apoptosis, DNA repair and immune responses. Dysregulation of the ubiquitin-proteasome system (UPS) is frequently observed in cancer cells, promoting tumourigenesis by stabilising oncoproteins or degrading tumour suppressors [3,4].

Recent studies have shown that manipulation of the ubiquitination pathway could provide new therapeutic avenues for cancer treatment. There is growing evidence for the potential of targeting many of the enzymes involved in ubiquitination, in particular deubiquitinating enzymes (DUBs) [4]. DUBs are responsible for removing ubiquitin from target proteins, counteracting the action of ubiquitin ligases, which can affect protein degradation and function. The process of ubiquitination plays a key role in immune cell function, especially in t-cell activation and regulation. By inhibiting DUB, the ability of t-cells to recognise tumour cells can be enhanced, thereby improving the body's ability to detect and eliminate cancer cells. It has been shown that the activity of DUB can be blocked through the use of specific small molecule inhibitors, which could potentially allow for the restoration of the normal ubiquitination and degradation processes of tumour-associated proteins. Therefore, this paper will focus on analysing the performance of small molecule inhibitors capable of inhibiting deubiquitinating enzymes in relevant experiments, verifying whether they can activate tumour suppressor pathways, thereby inhibiting cancer signalling and restoring the role of the immune system, with the ultimate goal of preventing tumour growth and metastasis. This strategy not only has the potential to synergise with existing targeted therapies, but also to broaden the scope of combination therapies, opening up new possibilities for more effective and personalised cancer treatments.

2. The Ubiquitination Pathway in Cancer

The ubiquitination pathway regulates protein stability and cellular signalling through post-translational modifications, which play a key role in cancer biology. The pathway is mediated by ubiquitin ligases and DUBs, which promote the addition and removal of ubiquitin from target proteins, respectively, and thus affect protein degradation by the proteasome. Notably, DUBs such as ubiquitin-specific protease7 (USP7) and USP2 have emerged as key players in the promotion of oncogenic processes by stabilising proteins such as p53 and survivin, which promote apoptosis resistance in cancer cells [5]. The complex relationship between DUB activity and tumourigenesis makes these enzymes promising targets for small-molecule inhibitors aimed at restoring normal apoptotic signals that are disrupted in cancer [4]. In addition, recent studies have highlighted the potential of specific inhibitors such as P5091 to reduce tumour cell viability while enhancing anti-tumour immunity [6]. This growing knowledge highlights the therapeutic promise of targeting the ubiquitination pathway in cancer treatment, providing new strategies to combat tumour resistance.

2.1. Mechanisms of Ubiquitination and Deubiquitination

Mechanisms of ubiquitination and deubiquitination play key roles in regulating protein stability, signalling pathways and cellular responses, particularly in cancer biology. Ubiquitination involves the covalent attachment of ubiquitin molecules to target proteins, usually signalling them through degradation by the proteasome. This post-translational modification is coordinated by E3 ligases, which are essential for substrate specificity and attachment of ubiquitin to the target protein. In contrast, DUBs remove the ubiquitin chain, thereby rescuing proteins from degradation and affecting various cellular processes, including apoptosis and DNA repair [5]. Moreover, DUBs such as USP7 and USP2 have emerged as key regulators of tumourigenesis, stabilisation of oncogens and promotion of cancer cell survival [4,7]. Recent studies have highlighted the therapeutic potential of small molecule inhibitors targeting these pathways, aiming to restore homeostasis in cancerous tissues by enhancing ubiquitination and reducing oncogenic signals [8]. By elucidating these dynamic

DOI: 10.54254/2753-8818/90/2025.GU20971

regulatory mechanisms, researchers aim to develop innovative strategies that target the complexity of the UPS. These advances offer promising avenues for overcoming resistance to current therapies.

2.2. Key Factors: Ubiquitin Ligases and DUBs

Ubiquitin ligases and DUBs play a key role in cellular regulation, especially in the context of cancer, where their dysregulation can lead to tumourigenesis. Ubiquitin ligases promote the addition of ubiquitin molecules to target proteins, labelling them for degradation or altering their function, while DUBs reverse this modification, thereby maintaining protein stability and cellular homeostasis. The balance between these two enzyme types determines cellular outcomes, including proliferation, apoptosis, and DNA repair mechanisms [8].

USPs are the largest and most well-studied family of DUBs that catalyse the removal of ubiquitin from target proteins. USPs are characterised by the presence of a conserved catalytic structural domain, termed the USP structural domain, which contains a highly conserved active site cysteine residue that plays a key role in the hydrolysis of the isopeptide bond between ubiquitin and the substrate protein. The USP catalytic domain is composed of a combination of β-strands and α-helices that form a structure capable of recognizing and binding to the C-terminus of ubiquitin, where the isopeptide bond is located. The catalytic cysteine residue within the USP domain attacks the isopeptide bond, releasing free ubiquitin and leaving the substrate protein either unmodified or with a reduced level of ubiquitin modification.

USPs have a variety of biological functions, due in large part to their ability to regulate the dynamics of protein ubiquitination. usps can prevent proteasomal degradation of specific proteins by removing the ubiquitin chains that normally mark them for disruption. For example, specific dubs such as USP7 are associated with the maintenance of mutant p53 levels in colorectal cancer, promoting cancer stem cell properties [7]. In addition, inhibition of dubs such as P5091 can improve the efficacy of existing treatments by inducing apoptosis in cancer cells that are resistant to conventional therapy [5]. As ongoing studies elucidate their roles, targeting these enzymes offers a promising avenue for developing new cancer therapies [9].

2.3. Impact of Ubiquitination on Tumor Progression and Immune Evasion

The complex role of ubiquitination in tumour progression and immune evasion highlights its importance in cancer biology and therapy. By regulating protein degradation and cell signalling, ubiquitination orchestrates key processes including apoptosis and cell proliferation. dub becomes a key player in this context; for example, oncogenic proteins such as p53 and survivinin can be stabilised through dub activity, contributing to tumour growth and treatment resistance [5]. Specific DUBs can act as oncogenes or tumour suppressors, leading to dysregulation of the immune response. For example, USP7 inhibits the function of immune checkpoint proteins and enhances tumour cell evasion of immune surveillance [4]. In addition, targeting DUB with small molecule inhibitors can improve the immunosuppressive TME, improve t-cell function and stimulate anti-tumour activity [9]. As research progresses, the therapeutic potential of DUB inhibitors in reversing immune evasion and mitigating cancer progression becomes increasingly apparent, paving the way for new cancer treatment strategies.

3. Small Molecule Inhibitors Targeting Ubiquitination

The quest for small molecule inhibitors that target ubiquitination is becoming increasingly important in the field of cancer therapy. This is because these compounds can modulate key cellular processes involved in tumourigenesis. For example, the USP2 inhibitor ML364 has been shown to sensitise cancer cells to experimentally induced apoptosis by promoting the degradation of the anti-apoptotic

DOI: 10.54254/2753-8818/90/2025.GU20971

protein survivin, which is frequently overexpressed in various malignancies. In addition, the USP7 inhibitor P5091 not only reduces tumour growth in colorectal cancer models, but also enhances the immune response, making it a promising candidate for combination therapy with immune checkpoint inhibitors [6]. In addition, findings suggest that targeting dubs such as USP7 and USP2 can disrupt the self-renewal and survival pathways of cancer cells, suggesting that small molecule inhibitors have the potential to serve as translational agents in cancer therapy. Thus, the development of selective inhibitors is a key strategy for cancer therapeutic intervention using the UPS [4,10].

3.1. Overview of Small Molecule Inhibitors and Their Mechanisms

Small molecule inhibitors have emerged as a key drug for targeting specific pathways involved in cancer progression, particularly during ubiquitination. By selectively inhibiting DUBs, these compounds can alter the stability and activity of key oncogenic and oncostatic proteins. For example, inhibitors such as ML364 target USP2 and have been shown to down-regulate survivin, thereby enhancing apoptosis in cancer cells through test activation, highlighting the significance of DUB inhibition in overcoming therapeutic resistance [8]. In addition, drugs like P5091 engage USP7, reducing its stabilising effect on mutant p53 and promoting anti-tumour immunity [5,6]. This subtle targeting of cancer-specific mechanisms, particularly through modulation of the UPS, offers the opportunity for personalised therapeutic strategies that exploit unique DUB interactions to evade traditional resistance mechanisms [4,9]. As research progresses, the potential of these small molecule inhibitors continues to expand with promising innovative therapeutic solutions.

3.2. Case Study: P5091 as a USP7 Inhibitor in Cancer Therapy

P5091 is a selective inhibitor of USP7, and its case study highlights its remarkable potential in cancer therapy, particularly for tumours that exhibit resistance to conventional treatments.P5091 inhibits tumour growth and promotes apoptosis in a wide range of cancer cell lines, including those with mutations in p53, which often lead to therapeutic resistance. The compound effectively destabilises USP7s against key oncogenic proteins and increases the degradation of substrates such as HDM2, ultimately restoring the activity of the tumour suppressor p53. In preclinical models, p5091 not only reduced tumour size, but also enhanced anti-tumour immunity by modulating pro-inflammatory cytokine levels and reducing the regulatory T-cell population. In addition, the study demonstrated that the effects of p5091 are not solely dependent on the status of p53, suggesting a broader applicability in combination with other therapies, thus making it a promising candidate in the development of cancer therapies [10,11].

3.3. Potential of Dual-target Inhibitors in Enhancing Treatment Efficacy

The exploration of dual-targeted inhibitors in cancer therapy has revealed great potential to improve efficacy by simultaneously modulating multiple pathways critical for tumour growth and survival. By specifically targeting deubiquitinating enzymes (DUB) and other oncogens, these small molecule inhibitors can disrupt important regulatory mechanisms, thereby increasing apoptosis and reducing cancer cell resistance [12]. For example, inhibition of USP2 along with inhibition of survivin is expected to sensitise cancer cells to TRAIL-mediated apoptosis, highlighting the importance of targeting proteins involved in apoptosis. Furthermore, the fact that DUBs are both oncogenes and tumour suppressors highlights their strategic importance in cancer pathways, suggesting that a dual-target approach can be effective in mitigating the complexity of tumour biology. Furthermore, studies with P5091 have shown that dual suppression enhances anti-tumour immunity while reducing tumour growth, particularly in models resistant to conventional therapies. This multifaceted approach reflects

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a promising direction for future cancer therapy, providing a powerful framework for overcoming drug resistance and improving patient prognosis [13].

4. Therapeutic Implications and Future Directions

The therapeutic significance of targeting DUBs in cancer therapy is far-reaching, especially in the context of developing small molecule inhibitors. Inhibition of specific DUBs, such as USP2, has demonstrated the potential to sensitise cancer cells to apoptosis, particularly through mechanisms involving the anti-apoptotic protein survivin, as described in [5]. In addition, it has been shown that USP7 inhibitors such as P5091 not only inhibit tumour growth, but also enhance anti-tumour immunity by modulating cytokine profiles and regulatory T-cell populations [6]. The multifaceted roles of DUBs in tumourigenesis imply that their inhibition may be effective in overcoming resistance to conventional therapies, supporting the discovery of a DUB deregulation linked to cancer stem cell characteristics and proliferation. Future directions in this field should prioritise the development of selective and potent DUB inhibitors, with an increasing focus on combination therapies that synergise with existing immunotherapy strategies [4].

4.1. Current Clinical Applications of Small Molecule Inhibitors in Oncology

The integration of small molecule inhibitors in oncology has facilitated significant advances in cancer therapy by targeting specific nodes in the ubiquitination pathway. These inhibitors, such as P5091, which targets USP7, have gained attention for their ability to enhance the efficacy of existing therapies while attenuating mechanisms of drug resistance in various malignancies. For example, P5091 has shown promising results in preclinical models of colorectal cancer by stabilising mutant p53 and inhibiting tumour stem cell properties, thereby inhibiting tumour growth [5]. In addition, inhibitors such as ML364 effectively down-regulate survivin, sensitizing cancer cells to test-mediated apoptosis while preserving normal cells. The multifunctionality of these inhibitors allows them to interact with multiple oncogenic pathways, thus providing an opportunity to improve therapeutic outcomes in a variety of cancers [4]. As research continues to elucidate the role of deubiquitinating enzymes in tumourigenesis, the clinical applicability of these small molecule inhibitors will soon be expanded, offering the promise of more targeted cancer therapy [6].

4.2. Challenges in Developing Selective Inhibitors for DUBs

The development of selective inhibitors of DUBs faces a considerable challenge in the context of targeted cancer therapy. One of the main difficulties is the structural similarity shared by different DUBs, which complicates the design of inhibitors that can selectively bind to specific enzymes without affecting others. This non-selectivity often leads to off-target effects that result in unexpected toxicity, as many dubs regulate essential cellular processes. In addition, the dynamic nature and complex biological interactions of dubs contribute to the complexity of developing inhibitors that provide therapeutic benefits without disrupting normal cellular function [14]. Advances in the study of DUB substrate interactions and their regulatory mechanisms have been crucial in overcoming these challenges, paving the way for more potent and selective drugs in cancer therapy. Ongoing research in this area is therefore critical for the discovery of innovative strategies that can improve the clinical viability of DUB inhibitors [15].

4.3. Future Research Directions and the Promise of Combination Therapies

Future research directions in the area of combination therapy highlight the great potential for combining small molecule inhibitors targeting DUBs with existing cancer therapies to enhance

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therapeutic efficacy. As studies reveal the multifaceted roles of dubs such as USP2 and USP7 in regulating apoptosis and cell proliferation, they highlight the promise of using inhibitors such as ML364 and P5091 in combination with modalities such as experimental and immune checkpoint inhibitors to overcome drug resistance. Further exploration of the mechanistic pathways of dub has revealed not only their status as oncogenes, but also their tumour suppressor functions, hence the need for precise therapeutic strategies targeting specific cancer types. In addition, targeting USP7 has been shown to improve immune responses and may act synergistically with immunotherapy to improve overall treatment outcomes. This integrative approach is likely to pave the way for new therapeutic frameworks and facilitate the development of more effective cancer management strategies [6,8].

5. Conclusion

The growing acceptance of small molecule inhibitors in the cancer ubiquitination pathway highlights their potential as transformative agents in oncology. As detailed herein, these inhibitors target specific DUBs, such as USP2 and USP7, which have been implicated in the regulation of apoptosis and cancer cell proliferation. The dual roles of DUBs as oncogenes and tumour suppressors underscore the complexity of their involvement in cancer biology, making them compelling therapeutic targets. Furthermore, the demonstrated therapeutic effects of compounds such as P5091 in reducing tumour growth and enhancing immune responses consolidate promising avenues for DUB inhibition in combination with existing therapies. Future research should continue to focus on elucidating the exact mechanisms of these inhibitors, as well as determining their selective impact on different cancer types, thus paving the way for more effective therapeutic regimens that exploit the complexity of the UPS.

In conclusion, exploring targeted inhibition via small molecule inhibitors in the cancer ubiquitination pathway has great potential to enhance therapeutic outcomes. By specifically disrupting the activities of deubiquitinating enzymes (DUB) and ubiquitin ligases, these molecules can restore the delicate balance of protein homeostasis that is often disrupted in malignant tumours. This targeted approach not only provides a way to circumvent the resistance mechanisms inherent in many conventional cancer therapies, but also allows for selective modulation of key oncogenic processes. As research progresses, the development of selective inhibitors promises to minimise off-target effects, thereby reducing toxicity and improving the overall efficacy of treatment. By leveraging our understanding of the UPS, targeted inhibition strategies could provide a powerful arsenal in the fight against cancer, ultimately leading to improved patient prognosis and a more personalised approach to cancer treatment.

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