Acta Pharmaceutica Sinica B 2026;16(3):1786−1789

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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HIGHLIGHT

Phosphatases: An undervalued class of antitumor drug targets

KEY WORDS

Phosphatase;
Antitumor drug targets;
PTPN2/PTPN1 inhibitor;
CDC25A;
Cryo-EM structure

1. The importance and drug design challenges of phosphatases as antitumor targets

Phosphatases are enzymes that remove phosphate groups from proteins and other molecules, playing a critical role in regulating various cellular processes. They act as key switches in cell signaling pathways, controlling cell cycle progression, gene expression, and cell growth[1,2]. In cancer, phosphatases can act as tumor suppressors or promoters. For example, PTPN2 and PTPN1 negatively regulate inflammation and immune cell function. Their loss in tumors or immune cells can enhance tumor immunoevasion and create an immunosuppressive microenvironment[3]. The CDC25 phosphatase family (CDC25A, B, and C) regulates cell cycle transitions by activating CDKs through dephosphorylation. CDC25A is particularly important for CDK2−cyclin A and CDK2−cyclin E activation at the G1/S transition and plays a role in G2/M phase transitions. The abnormal expression or function of these phosphatases is associated with uncontrolled cell proliferation, apoptosis resistance, and altered tumor microenvironments, making them promising targets for cancer therapy[4,5].

Beyond PTPN2/PTPN1 and CDC25A, other phosphatases such as SHP2 (PTPN11), PP2A, and DUSP family members are also implicated in cancer. SHP2, a non-receptor tyrosine phosphatase, drives oncogenic RAS/MAPK signaling and is targeted by allosteric inhibitors (*e.g.*, SHP099) in clinical trials[6,7]. PP2A, a serine/threonine phosphatase, acts as a tumor suppressor but is often dysregulated in cancers; its reactivation (*e.g.*, *via* FTY720 derivatives) shows therapeutic potential[8]. DUSPs (*e.g.*, DUSP6) regulate MAPK pathways and tumor immune evasion, offering additional targeting opportunities[9]. These examples further highlight the broad relevance of phosphatases in cancer therapy.

However, developing drugs targeting phosphatases is challenging. One major challenge is the conserved nature of phosphatase active sites. The active sites of phosphatases are highly conserved, which makes it difficult to design selective inhibitors. For instance, the high homology between the active sites of PTPN2 and PTPN1 can lead to cross-reactivity, affecting drug selectivity and efficacy[5]. Another challenge is the complex substrate specificity of phosphatases. Phosphatases can act on a wide range of substrates, and their specificity is influenced by multiple factors, including substrate sequences, the 3D structural complementarity between the phosphatase and substrate, and the cellular environment. This complexity complicates the design of drugs that can precisely target specific phosphatase-substrate interactions. Additionally, phosphatases undergo diverse conformational changes during their function, which are crucial for their activity and interactions with other proteins. However, this diversity in conformational changes makes it difficult for drugs to stably bind to phosphatases, affecting the binding affinity and inhibitory effects of the drugs[3-5].

2. Discovery and antitumor immune research of PTPN2/PTPN1 inhibitor ABBV-CLS-484[3]

Immunotherapy has revolutionized cancer treatment, but many patients do not respond to current immunotherapies. PTPN2 and PTPN1 are tyrosine phosphatases that negatively regulate inflammation and immune cell function. Genetic ablation of PTPN2 or PTPN1 in tumor cells enhances the response to immunotherapy. ABBV-CLS-484 is a first-in-class, orally bioavailable, and potent PTPN2/PTPN1 active-site inhibitor. ABBV-CLS-484 was discovered through extensive drug design and screening efforts

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.11.021

(Structure-based drug design and medicinal chemistry optimization). It enhances the response to interferons and promotes the activation of several immune cell subsets *in vitro*.

In vitro studies showed that ABBV-CLS-484 treatment amplified the response to interferon and promoted the activation and function of immune cells. In mouse models of cancer resistant to PD-1 blockade, ABBV-CLS-484 monotherapy generated potent antitumor immunity. ABBV-CLS-484 was found to inflame the tumor microenvironment and promote the function of natural killer cells and $CD8^+$ T cells by enhancing JAK−STAT signaling and reducing T cell dysfunction. In terms of quantitative results, the treatment with ABBV-CLS-484 resulted in a significant increase in the frequency of tumor-infiltrating $CD45^+$ and $CD8^+$ cells in B16 and KPC tumors, comparable to changes elicited by anti-PD-1 treatment. Additionally, ABBV-CLS-484 treatment led to a significant increase in the ratio of M1 macrophages to M2 macrophages and the ratio of monocytes to myeloid-derived suppressor cells (MDSCs). Furthermore, ABBV-CLS-484 enhanced the activation and cytokine production of primary mouse T cells *in vitro*. It increased the levels and duration of LCK and FYN phosphorylation in both resting and anti-CD3-stimulated mouse T cells. ABBV-CLS-484 treatment also increased the proliferation of T cells activated with suboptimal anti-CD3/CD28 stimulation.

In terms of antitumor effects, ABBV-CLS-484 demonstrated significant antitumor activity across multiple syngeneic and metastatic mouse models. In the B16 melanoma model, treatment with ABBV-CLS-484 resulted in a statistically significant reduction in tumor volume compared to untreated controls, with a *P*-value of less than 0.0001. In the KPC pancreatic cancer model, ABBV-CLS-484 treatment also showed a significant reduction in tumor growth, with a *P*-value of less than 0.0001. Similarly, in the 4T1 mammary carcinoma and EMT-6 breast carcinoma models, ABBV-CLS-484 treatment led to significant tumor regression and increased survival rates. In the CT26 colon cancer model, the combination of ABBV-CLS-484 and anti-PD-1 therapy further enhanced treatment efficacy, with a *P*-value of less than 0.0001. In the B16 pulmonary metastasis model, ABBV-CLS-484-treated mice developed no detectable disease, achieving a 100% survival rate, while untreated and anti-PD-1-treated mice developed lung metastases by Day 10. In the 4T1 orthotopic breast cancer model, ABBV-CLS-484 treatment reduced the number of lung metastases. These results highlight the potential of ABBV-CLS-484 as a novel antitumor agent.

3. Cryo-EM structure of the CDK2−cyclin A−CDC25A complex[5]

Cell cycle progression is tightly regulated by a family of proteins known as cyclin-dependent kinases (CDKs). CDKs require association with their cyclin partners for activity and are controlled by protein binding and posttranslational modifications. The CDK2−cyclin A−CDC25A complex plays a crucial role in cell cycle regulation. The cryo-EM structure of this complex, determined at 2.7 Å resolution, provides detailed insights into the overall complex architecture and key protein−protein interactions. The structure reveals a CDC25A C-terminal helix that is critical for complex formation. Sequence conservation analysis suggests that CDK1/2−cyclin A, CDK1−cyclin B, and CDK2/3−cyclin E are suitable binding partners for CDC25A, while CDK4/6−cyclin D complexes are unlikely to be substrates for CDC25A.

The CDK2−cyclin A−CDC25A complex is an approximately 86 kDa trimer. In this complex, CDK2 maintains its characteristic bilobed structure, consisting of a β-sheet N-terminal lobe and a predominantly α-helical C-terminal lobe. The structure of cyclin A is highly conserved, composed of two helical domains (N-CBF and C-CBF) that bind both lobes of CDK2, forming an extensive protein−protein interface. The CDC25A catalytic domain structure is largely consistent with the monomeric crystal form, featuring an α/β domain with a central five-stranded parallel β-sheet enclosed by five α-helices. The mutated catalytic residue (Cys431Ser) initiates the conserved PTP C(X)5R loop between a central β-strand (β4) and an α-helix (α4), creating a shallow active site that recognizes CDK2 pTyr15. The CDC25A catalytic domain bridges the bilobed structure of CDK2, binding to the opposite face of cyclin A to form an extensive but discontinuous interface of approximately 1260 $Å^2$.

The study also involved hydrogen-deuterium exchange mass spectrometry (HDX-MS) analysis of the CDK2−cyclin A−CDC25A complex, comparing monomeric CDC25A against the trimeric complex. The analysis highlighted a region of CDC25A that is significantly protected upon complex formation. Specifically, peptide 1 (VRERDRLGNEYPKLHYPEL, residues 445−463) showed enhanced protection in the trimeric complex. Within the CDK2 N-terminal lobe, a peptide spanning residues 38−51 (DTETEGVPSTAIRE) showed significant protection in the ternary complex. This peptide includes the PSTAIRE (αC) helix and the preceding loop, which bind the CDC25A C-terminal helix (α6).

The HDX-MS data did not reveal any significant protection for the C-terminal domain of CDC25A, likely due to the relatively distant nature of the interaction with the CDK2−cyclin A interface and the structurally dynamic behavior of this region. The refined atomic displacement parameters of atoms within the helix reveal high temperature factors across the helix, except for residues 512−517 directly involved in contacts to CDK2 and cyclin A.

Surface plasmon resonance (SPR) experiments were conducted to explore the requirement for cyclin A in complex formation. The affinity of CDC25A(C431S) for pT160CDK2−cyclin A was significantly greater than that for monomeric pY15pT160CDK2 and pT160CDK2. Despite making a few interactions with CDC25A, cyclin A plays a significant role in facilitating the formation of the CDK2−cyclin A−CDC25A complex by enforcing changes in CDK2 conformation around the active site.

The study also investigated the role of the CDC25A C-terminal tail in binding to CDK2−cyclin A. Through homogenous time-resolved fluorescence (HTRF) assays, it was found that terminating the CDC25A construct at Glu495, and charge reversal mutations in the CDC25A C-terminal helix (R502E/K504E/R506E and K514E/R520E) abrogated binding, underscoring the importance of the C-terminal helix for trimeric complex formation.

The structure of CDK2 bound to KAP and CKS1 was compared with the CDK2−cyclin A−CDC25A complex. The GDSEID motif in CDK2 was found to be a mutual binding site for these proteins, conferring selectivity through interactions with different CDK2 residues. The GDSEID-αG helix motif provides a hotspot for protein binding, offering a mechanistic explanation for the identification of yeast cell cycle mutants within this sequence.

Sequence conservation analysis of critical residues involved in CDC25A recruitment suggests that CDK1/2−cyclin A, CDK1−cyclin B, and CDK2/3−cyclin E complexes are suitable binding partners for CDC25A. In contrast, CDK4−cyclin D complexes appear to be unlikely substrates for CDC25A based on sequence conservation. AlphaFold Multimer models were used to assess the potential for CDK4−cyclin D3 to bind to CDC25A. The models consistently predicted that CDC25A binds to the cyclin

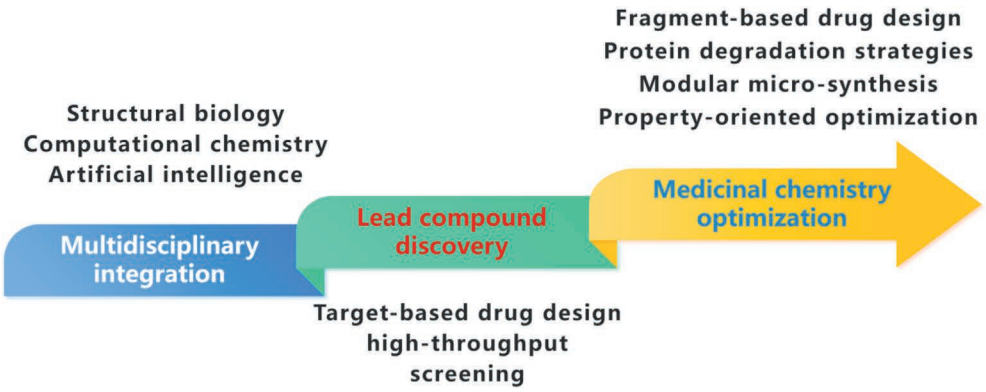

Figure 1 Research on anti-tumor drugs targeting phosphatases in the context of multidisciplinary integration.

subunit with no interaction with CDK4, supporting the comparative sequence analysis.

4. Summary and outlook

Phosphatases are essential regulators of cellular processes and have emerged as promising targets for antitumor therapy. The development of ABBV-CLS-484 and the structural analysis of the CDK2−cyclin A−CDC25A complex highlight the potential of phosphatases as targets for cancer treatment. Despite the challenges in drug design, advances in structural biology and computational methods are providing new opportunities for the development of phosphatase-targeting drugs.

The discovery of ABBV-CLS-484 represents a significant step forward in the development of PTPN2/PTPN1 inhibitors for cancer therapy. The preclinical data demonstrate that ABBV-CLS-484 is a potent and selective inhibitor with promising antitumor activity in various mouse models. The ongoing clinical trials will provide crucial information on its safety and efficacy in humans[3].

The cryo-EM structure of the CDK2−cyclin A−CDC25A complex offers valuable insights into the mechanism of CDC25A in cell cycle regulation. This structural information could guide the development of novel anticancer drugs targeting the CDC25 phosphatases. The identification of key interactions and binding sites may facilitate the design of inhibitors that can disrupt the function of CDC25A in cancer cells[5].

It's worth noting that the author's research team[10,11] had previously successfully applied modular microsynthesis and rapid screening techniques to the discovery of novel CDC25 inhibitors. These techniques, known for their versatility, have been effectively used across numerous targets and are expected to play a significant role in the discovery of inhibitors for other phosphatase targets as well[12-14]. In addition, protein degradation technologies, recognized as a transformative force in drug discovery in the 21st century[15], are also anticipated to have significant potential in the development of antitumor drugs targeting phosphatases[16].

Looking to the future, continued research into the biology of phosphatases and the development of innovative drug design strategies (including high-throughput screening, fragment-based drug design, and property-oriented structural optimization) will be essential to overcome the challenges in targeting these enzymes (Fig. 1). The potential of phosphatases as antitumor targets remains largely untapped, and further exploration could lead to the discovery of new therapeutic agents for the treatment of cancer. With the right scientific approach and collaborative efforts, phosphatase-targeting therapies may soon become an integral part of the oncology treatment landscape.

Acknowledgments

The authors are supported by the Key Research and Development Program, Ministry of Science and Technology of the People's Republic of China (No. 2023YFC2606500).

Author contributions

Yuning Song conceived the idea. Mei Wang, Peng Zhan, and Yuning Song wrote the draft manuscript, then revised the manuscript. All authors have read and approved the submission of the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Received 29 June 2025
Received in revised form 20 July 2025
Accepted 31 July 2025