Breast Cancer: Targets and Therapy

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REVIEW

Research Progress of PROTAC-Degraded CDKs in the Treatment of Breast Cancer

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Abstract: Breast cancer (BC) is the most common type of cancer among women worldwide. A large number of studies have found that the high expression or dysregulation of cyclin-dependent protein kinases (CDKs) is closely associated with breast cancer. For example, the CDK4/6-Rb axis is involved in the G1/S phase transition of the cell cycle and plays an important role in BC; CDK1 and its associated cyclin are commonly involved in mitotic progression, and increased expression of CDK1-associated cyclin has been observed in BC; loss of CDK12 significantly ameliorates triple-negative breast cancer. CDKs are one of the major families within the group of PROteolysis Targeting Chimeras (PROTACs)-degraded kinases. PROTAC is a potent technology for protein-targeted degradation, whose molecules consist of the ligand of the Protein of Interest (POI), the ligand of the E3 ubiquitin ligase (E3), and a Linker. After binding to POI, PROTAC can recruit E3 to ubiquitinate POI via ubiquitin-proteasome mediated degradation. In this review, we summarize relevant research results and review that PROTAC can effectively inhibit the proliferation of breast cancer cells by inducing ubiquitination of CDK1, CDK4/6, CDK9, CDK12/13 and their subsequent degradation by proteasomes, which is expected to be a novel approach for the treatment of breast cancer.

Keywords: breast cancer, ubiquitin, PROTAC, CDKs

Background

According to the latest statistics from the American Cancer Society, breast cancer(BC) remains the most common malignant tumor in women worldwide, replacing lung cancer, and its incidence is slowly increasing each year.[1] The report from the International Agency for Research on Cancer indicates that the global number of new breast cancer cases is projected to exceed 3 million by 2040.[2] With the in-depth research on cancer mechanisms and the continuous updating of treatment methods, the survival rate of high incidence cancers such as BC has been improved, but cancer is still the leading cause of death among middle-aged and elderly people, and BC is the leading cause of cancer death among women.[3] These concerning trends highlight the urgency of developing more effective strategies to target BC.

Originally characterized as serine/threonine-specific protein kinases, CDKs are central to the regulation of the eukaryotic cell cycle.[4] Cyclin acts as an activator for CDKs and is an integral part of the cell cycle machinery.[5] CDK monomers are normally inactive, and they drive the eukaryotic cell cycle by combining with the corresponding cyclin to form a heterodimer with activity.[6] In addition to CDK1, CDK2, CDK4, and CDK6, which regulate the cell cycle, an important sub-branch of the family, CDK7, CDK9, CDK12, and CDK13, regulates transcription.[7] CDK7, CDK9, CDK12, and CDK13 directly phosphorylate the C-terminal structural domain (CTD) of RNA Pol II, thereby regulating different stages of transcript production. Mounting evidence has revealed a strong association between CDKs and cancer development. For instance, Liu et al[8] found that CDK2 was a potential diagnostic and prognostic biomarker and novel tumor immune environment sign for glioma patients. And numerous studies have demonstrated that CDKs are also inextricably linked to various types of breast cancer, such as CDK1, which is involved in the development of TNBC as a key cell cycle regulator controlling G2/M;[9] the CDK4/6-Rb axis, which has been shown to increase the rate of

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progression from the G1 to the S stage, especially in estrogen-receptor-positive breast cancer;[10,11] and CDK9, which promotes the epithelial-mesenchymal transition (EMT) induces cancer stem cells and is highly expressed in breast cancer.[12] Therefore, targeting CDK has been identified as a promising approach for cancer therapy.[13,14] CDKs are one of the major kinase families effectively degraded by PROTAC compounds,[15,16] therefore, the application of PROTAC technology to directly degrade CDKs for the treatment of breast cancer provides a new solution for the treatment of breast cancer.

PROTAC technology is an effective tool for endogenous protein degradation developed in recent years. PROTAC is a bifunctional small molecule compound containing two different ligands in its structure: one is an E3 ligand and the other is a ligand that binds to POI. These two ligands are connected to each other by Linker, forming a ternary compound: target protein ligand-Linker-E3 ligand. This special structure allows PROTAC to bind to both the target protein and the E3 ligase, thus targeting the degradation of proteins that are difficult to inhibit by conventional drugs.[17,18]

Mechanism of Action of PROTAC

Ubiquitin and Its Mechanism of Action

There are many pathways of protein degradation, among which the ubiquitin-proteasome system (UPS) is the main pathway of protein degradation in cells, which maintains protein homeostasis by ubiquitin labelling of proteins to be degraded, and then recognition and degradation of these labelled proteins by the proteasome. Its ability to specifically degrade proteins involved in various metabolic activities is important for maintaining cellular protein homeostasis and regulating many cellular processes such as gene transcription, DNA pairing, cell cycle control and apoptosis.[19,20] UPS is ATP-dependent and consists of two steps: polyubiquitination of target proteins and proteolytic hydrolysis of polyubiquitin by the 26S protein hydrolase complex.[21] Ubiquitin is a small regulatory protein, contains seven lysine residues and an N-terminal methionine residue, each of which can be attached to another ubiquitin with the function of labeling proteins for degradation.[22] Ubiquitination is a post-translational modification that involves the binding of ubiquitin to lysine residues of other proteins. This modification is mediated by the sequential action of E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase. First ubiquitin is activated by E1 in the presence of ATP, then it binds to E2, and finally it is linked to the lysine residues of the target protein via E3, forming a ubiquitin-protein covalent linkage. The ubiquitin-tagged protein is then recognized by the proteasome and transported to the enzyme factory for degradation, and ubiquitin can be recycled repeatedly[23,24] (as shown in Figure 1). PROTAC is a drug design strategy based on the ubiquitin-proteasome system for targeted protein degradation in cancer therapy. Further, CRISPR-Cas9 is a genome editing tool.[25] CRISPR screens have been used to identify essential genes, genes involved in cancer metastasis and tumor growth, as well as drug targets and mechanisms of resistance.[26] For example, Kumarasamy et al[27] identified CDK2 loss as a mechanism of resistance to CDK2 inhibitors by CRISPR screens in a breast cancer model. In addition, knockdown of CDK2 by CRISPR-Cas9 confirmed that CDK2 deletion reversed CDK2 inhibitor-induced G2/M block and restored cell proliferation. In summary, CRISPR screens provide insights into the mechanisms of cancer drug resistance, develops more effective therapeutic strategies, and brings new breakthroughs for overcoming cancer drug resistance.

E3 Ubiquitin Ligase Ligands

E3 ligand is an important component of PROTAC, which is responsible for recruiting E3 in the human body thereby eliciting ubiquitin molecules, tagging the whole ternary complex for ubiquitin degradation and transporting it to the enzyme factory for degradation of the target protein. More than 600 E3s have been identified in the human genome, but only a few of them have been used in the design of PROTACs. Currently used to develop E3 ligands are Mouse double minute 2 homolog (MDM2), inhibitor of apoptosis proteins (IAPs), von Hippel- Lindau (VHL), cereblon(CRBN), and others.[28]

MDM2-PROTAC

The earliest MDM2-PROTAC utilized a derivative of Nutlin-3a as an E3 ligand to degrade the androgen receptor (AR).[29] MDM2-PROTACs selectively bind to the p53 site on the surface of MDM2, which can stabilize the p53 protein and

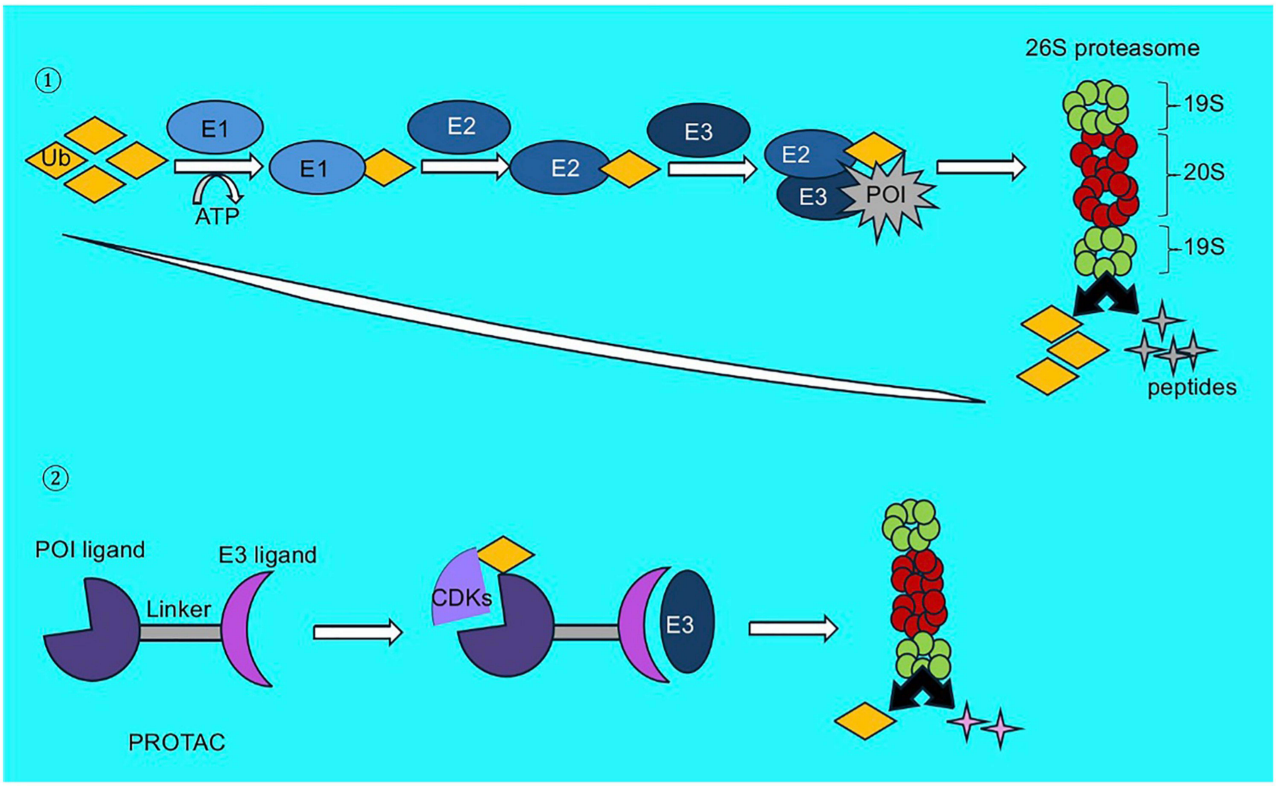

Figure 1 ① Ubiquitin-activating enzyme (E1) activates ubiquitin (ub) in the presence of ATP, and the activated ub is transferred to ubiquitin-conjugating enzyme (E2), and E3 ubiquitin ligase (E3) transfers the ub from E2 to lysine residues of target proteins to form ubiquitin chains. Eventually, the ubiquitin chain is recognized as a signal by the 26S proteasome. The target protein is recognized and unfolded by the 19S regulatory particle of the proteasome. The unfolded protein passes through the proteasome channel into the 20S core particle, proteases within the 20S core particle degrade the target protein into short peptides. Ub is then released from the degradation product and recycled. ② The target protein ligand of PROTAC binds to the target protein, and the E3 ubiquitin ligase ligand simultaneously binds to E3 ubiquitin ligase to form a ternary complex. The E3 ubiquitin ligase transfers the ubiquitin to the target protein, labeling it for degradation. The ubiquitinated target protein is recognized and degraded by the 26S proteasome.

degrade target proteins at the same time, and exhibit better anticancer activity. However, MDM2-binding ligands are not easy to synthesize, which leads to their limited application.

IAP-PROTAC

Early IAP-PROTACs utilized Bestatin derivatives as E3 ligands to degrade cellular retinoic acid binding protein-1/2 (CRABP-1/2).[30] Then later, IAP-PROTACs can also be used to degrade target proteins such as BRD4, AR, and ERα.[31,32] This series of PROTACs is also known as SNIPERs (specific and nongenetic IAP-dependent protein erasers). Because certain tumor cells escape apoptosis by directing the upregulation of IAPs, SNIPERs can often degrade both IAPs and target proteins, thus exerting stronger anti-tumor effects.

VHL-PROTAC

The VHL gene is a tumor suppressor gene, and its protein product VHL can form the VHL-ElonginB/C-CUL2 (VBC) complex with ElonginB, ElonginC, and Cullin2 proteins, which belongs to the E3-ubiquitin protease system and mediates the degradation of a variety of proteins in the human body, including BRD4 protein,[33] ERRα, RIPK2, and ABL, among others.[34,35] The main advantage of VHL-PROTACs is their targeting specificity. In addition, the fact that the binding affinity of the E3 ligase of VHL to the ligand does not need to be high is also one of its advantages.

CRBN-PROTAC

CRBN is a component of the CRL4 E3 enzyme complex, and a common ligand is a duamine derivative. Since a series of imines have been identified as ligands for CRBN, more and more CRBN-PROTACs have been developed. The first CRBN-PROTAC molecule used Thalidomide as a CRBN ligand to induce the degradation of BRD2, BRD3, and

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BRD4.[36] CRBN-PROTACs can also degrade a variety of substrates such as BCL6, CDK8/9, PI3K, BTK, and ALK. Since CRBN is widely expressed in tumor cells and normal cells, CRBN-PROTACs have less tissue selectivity.

The two most dominant ligands are VHL and CRBN.[37] First, both are very easy to obtain, and they are non-toxic and relatively cheap. Secondly, they have good binding sites and can be connected to linkers, which facilitates the synthesis of PROTAC molecules. Thirdly, they are very versatile and can be used to degrade multiple targets with a certain degree of flexibility. Last but not least, the two E3 enzyme ligands are well recognized by E3 enzymes and can be easily linked to E3 enzymes to recruit ubiquitin for degradation of target proteins.[38,39] Because CRBN has a larger protein-binding surface than VHL, CRBN-PROTACs may degrade a larger range of target proteins with broader target adaptation than VHL-PROTACs. The molecular weight of CRBN-PROTACs is also lower compared to VHL-PROTACs, so CRBN-PROTACs are in a more suitable chemical space for oral absorption.

Linker Selection

Linker is the part that chemically connects the Protein of Interest (POI, also known as drug targets, they are proteins that are directly bound to and affect the function of a drug when it exerts a therapeutic or diagnostic effect in the body) ligand to the E3 ligand. Currently, most of the Linkers are composed of polyethylene glycol chains or pure carbon chains, and the use of other Linkers such as p-xylene has also been reported in the literature.[40] Moreover, different Linker lengths can affect the activity of PROTACs.[41] In some cases Linkers are not bystanders of the PROTAC ternary complex, they can also form connections with the surface of the target protein, which enhances the activity of PROTACs.[42] Since different PROTACs molecules have different structures, they all have an optimal Linker; in other words, different PROTAC molecules do not have exactly the same optimal Linker.

Mechanism of Action of PROTAC

The mechanism of PROTAC is to use the UPS system to ubiquitinate and degrade target proteins to inhibit tumor growth.[43,44] Firstly, after the PROTAC molecule enters the cell, the E3 ligand recruits E3, and the POI ligand recruits the target protein that it wants to degrade, which ultimately binds the target protein to E3 and further ubiquitinates the target protein.[45] The ubiquitinated target protein is finally transported to the enzyme factory to be recognized and degraded by the 26S proteasome. And like ubiquitin, PROTAC molecules are theoretically recyclable[29,30] (as shown in Figure 1). Therefore, PROTACs require only a small dose to accomplish degradation in tumor cells.

PROTAC Degradation of CDKs for Breast Cancer Treatment

PROTAC Degradation of CDK1 for the Treatment of Breast Cancer

CDK1 was the first CDK family member to be identified.[46,47] Different cyclins are required at different stages of the cell cycle, and different CDKs regulate the cell cycle by binding to the corresponding cyclin.[48,49] In late G2 and early M, cyclin A binds to CDK1 to promote M phase. CDK1 is the only CDK that binds to cyclin B, which accumulates in M phase, leading to activation of the cyclin B-CDK1 complex.[50] Activation of cyclin B-CDK1 signals the onset of mitosis[51,52] and mitosis requires cyclin A and cyclin B in complex with CDK1 to ensure its successful completion.[53,54] Until cyclin A and cyclin B are disrupted by the ubiquitin-proteasome system,[55,56] CDK1 activity is depleted at the onset.[57,58] In addition to binding to cyclin, full activation of CDK1 is also regulated by phosphorylation of threonine and tyrosine residues. Wee1 or Myt kinases phosphorylate CDK1 at the inhibitory sites tyrosine-15 and/or threonine-14 thereby inhibiting CDK1 activity. Subsequently CDK activating kinase (CAK) phosphorylates CDK1 at the activation site threonine-161. Finally phosphorylates the inhibitory site in the presence of the Cdc25 phosphatase allowing full activation of CDK1 to drive further progression of the cell cycle.[59] These phosphorylations induce conformational changes and enhance the binding of cell cycle proteins.[60,61] The expression of CDK1 in breast cancer tissues is significantly higher than that in normal breast tissues and correlates with histological grading, pathological type and lymph node metastasis of breast cancer.

Xue et al[62] designed a series of CST-based PROTACs (compounds 6a-6d) with different length junctions using celastrol(CST) as the target protein binding ligand and thalidomide as the E3 ubiquitin ligase binding ligand. The

antiproliferative activities of compounds (6a-6d) were evaluated in five different cell lines, namely, 4T1, U87MG, A549, MDA-MB-231 and HepG2, and it was found that compound 6a showed superior cell growth inhibition. And compound 6a could inhibit the proliferation and migration of breast cancer cells. Compound 6a can inhibit the growth of 4T1 cells by causing 4T1 cell cycle arrest in G0/G1 and G2/M phases. And its induced cell cycle arrest was mainly regulated by CDK.[63] The ubiquitination level of CDK1 was significantly upregulated in 4T1 cells treated with compound 6a, which directly binds to CDK1 to induce protein degradation.[64] Compound 6a also showed excellent in vivo antitumor efficacy in a 4T1 hormonal Balb/c mouse-based model with an acceptable safety profile and could be used as a potential chemotherapeutic agent against triple-negative breast cancer (TNBC). It provides a new strategy for the treatment of TNBC and other cancers.

PROTAC Degradation of CDK4/6 for the Treatment of Breast Cancer

CDK4 and CDK6 bind to cyclin D (cyclin D1, cyclin D2, and cyclin D3) when DNA synthesis occurs, and are particularly involved in driving the cellular transition from the G1 to the S phase of the cell cycle.[65,66] That is why CDK4 and CDK6 are usually considered as promoters of G1 progression. The tumor suppressor retinoblastoma (Rb) protein controls the critical transition from G1 to S phase. Rb controls early cytokinesis by binding to the E2F transcription factor to block the G1/S transition, and inactivation of Rb allows cytokinesis to continue. In G1 phase, multiple growth signals can lead to binding of cyclin D to CDK4/6, and the resulting phosphorylation of Rb leads to the release of E2F transcription factors, which increase transcription of the E2F target genes cyclin E1 and cyclin E2. Cyclin E then binds to CDK2 and activates, leading to hyperphosphorylation of Rb and phosphorylation of many other proteins, which completely deprives Rb of its inhibitory effect on E2F transcription factors, collectively driving irreversible S phase.[67,68] The typical function of hypophosphorylated Rb is to bind E2F transcription factors and consequently to sequester E2F transcription factor activity, thereby inhibiting cell entry into S phase.[69] In BC, aberrant activation of the Cyclin D-CDK4/6-Rb signaling pathway is a key mechanism leading to tumor cell proliferation.[70] Indeed, most PROTACs targeting CDK4/6 can disrupt both kinases because they share a similar structural fold and common ligands.[71]

The pivotal role of CDK4/6 in the tumorigenic pathway makes CDK4/6 an attractive and safe target for anticancer therapy.[72] There are a number of studies designing PROTACs that degrade CDK4/6 to treat cancer. Amarnath Natarajan et al[73] reported that Palbociclib-based PROTACs selectively degraded CDK6 while retaining CDK4. Niall A. Anderson et al[74] reported Palbociclib-based PROTAC degraded CDK4/6 with high binding affinity and degradation potency. In addition, PROTACs recruiting VHL and cIAP ligases were prepared and they also showed good degradation efficacy for CDK4/6. Christian Steinebach et al[75] led their team in the design of a Palbociclib-based (palbociclib, a CDK4/6 inhibitor) PROTAC and recruited different E3 ligases, ie, CRBN and VHL. The VHL-based PROTACs, compounds 27 and 34, had comparable inhibitory activity to palbociclib against the negative breast cancer cell line MDA-MB-231 cells, suggesting that this effect is mainly controlled through CDK 4/6 inhibition. CRBN- and VHL-based PROTAC were next compared for degradation of CDK4/6 in MDA-MB-231 cells, revealing that CRBN-based PROTAC (BSJ-03-123) and 27 were more effective than 34. And VHL-based PROTAC was stronger on CDK6 than on CDK4. Both 34 and palbociclib significantly impaired cell migration and 34 was slightly better than palbociclib. Zhao et al[76] reported that PROTAC: degradant 1 is made by linking pomalidomide (a drug used to treat certain types of cancer such as multiple myeloma and Kaposi's sarcoma) as a ligand for the E3 ligase to palbociclib as a target protein binding ligand. Treatment of MDA-MB-231 cells with degradant 1 resulted in efficient degradation of CDK4 and CDK6, which subsequently reduced Rb phosphorylation levels in a dose-dependent manner. All of these studies provide new strategies for the treatment of cancers such as breast cancer by designing CDK4/6 degraders, making PROTAC a possible new tool for cancer therapy in the future.

PROTAC Degradation of CDK9 for the Treatment of Breast Cancer

CDK9 is a key member of the transcriptional CDK subfamily[77] and is mainly involved in transcription elongation. Transcriptional CDKs are associated with cancer because they contribute to the transcription of genes that are considered oncogenic transcription factors (TFs), such as c-MYC.[78] CDK9 forms a positive transcription elongation factor B (p-TEFb) complex with cyclin T, which promotes transcription elongation and mRNA maturation by phosphorylate

the C-terminal structural domain (CTD) of RNA pol II.[79,80] In addition, the active p-TEFb/CDK9 complex is recruited by bromodomain-containing protein 4 (BRD4) and the super elongation complex (SEC) to enhance c-MYC transcription.[81,82] Inhibition of CDK9 blocks phosphorylation of RNA polymerase II by p-TEFb, leading to transcriptional repression and apoptosis.[12] Dysregulation of CDK9 and its pathway plays an important role in several cancers.[83] Similarly, the key transcriptional regulator CDK9 is frequently dysregulated in BC.[84] In addition, the ATP-binding pocket of CDK9 is more flexible and can accommodate larger ligands compared to other CDKs, making CDK9 an attractive target for anticancer drugs.[85]

Noblejas-López et al[79] used the CDK9 inhibitor SNS-032 as a target protein-binding ligand, ligated SNS-032 to a thalidomide derivative, which binds to the E3 ubiquitin ligase CRBN, and designed PROTAC——THAL-SNS-032. Using MDA-MB-231, HS578T, BT549, SUM149, and HCC3153 as triple-negative breast cancer cell lines; SKBR3, HCC1569, and HCC1954 as HER2-positive cell lines; and MCF7 and T47D as luminal A cell lines. Finally, BT474 served as a luminal B cell line. CDK9 protein levels were assessed in all cell lines and found to be increased in lumen A and lumen B compared to TNBC and HER2-positive isoforms. The use of THAL-SNS-032 showed high inhibitory activity in MCF7, T47D and BT474 cells and was able to degrade CDK9 in the cells and induce apoptosis.

Chen et al[86] screened four PROTAC utilizing CDK9 inhibitors as ligands for target proteins and CRBN as an E3 ubiquitin ligase in the PROTAC libraries of triple-negative breast cancer cell lines HCC1806 and HCC1937: L027, L055, L062, and L063. Treatment of two ERα-positive cell lines (MCF7 and T47D), two HER2-positive breast cancer cell lines (BT474 and SKBR3), and two triple-negative breast cancer cell lines (HCC1806 and HCC1937) with the four compounds revealed that L055 exhibited the strongest inhibitory activity. Moreover, L055 significantly inhibited the proliferation, cell cycle, colony formation and induced apoptosis of MCF7 and T47D in vitro. Two ERα-positive breast cancer organoid models were established, and L055 inhibited organoid and tumor growth. Finally, using a T47D breast cancer hormonal nude mouse model revealed that treatment with L055 significantly reduced tumor growth and led to CDK9 degradation. Treatment of MCF7 and T47D cells with L055 for 48 h revealed that L055 significantly down-regulated CDK9 and reduced the expression of downstream target genes such as c-Myc and Mcl-1, and that it had a significant inhibitory effect on the growth of ERα-positive breast cancer cells. L055 represents a potentially novel therapeutic agent for ERα-positive breast cancers and potentially other malignancies, offering new insights and potential for the treatment of breast cancers through PROTAC breast cancer provides new insights and evidence.

PROTAC Degradation of CDK12/13 for the Treatment of Breast Cancer

CDK12/13 are also transcription-associated CDKs. Among the CDK family members, CDK12 and CDK13 have the highest sequence homology and the largest molecular weights. CDK13 has a similar sequence and a similar biological function as CDK12, but little research has been done on CDK13.[87] Among all CDKs, only CDK12 is located on chromosome 17q12, which always contains oncogenic features and genetic alterations of various tumors.[88] Cyclin K is a cell cycle protein that interacts with CDK12.[89] CDK12/13 binds to cyclin K by phosphorylating the C-terminal structural domain (CTD) of RNA pol II to regulate gene transcription, which is considered a key step in the transition from transcription initiation to elongation.[90] CDK12 mutations, amplifications, fusions and deletions can be found in different human cancers.[91] Therefore, CDK12 can act as a biomarker and therapeutic target in different cancer types.[92,93] Recent studies have revealed some novel functions of CDK12 in cancer, especially breast cancer, by regulating a variety of biological activities including c-MYC expression, Wnt/β-linker protein signaling, ErbB-PI3K-AKT signaling and DNA damage response (DDR) signaling.[94,95] Therefore, CDK12/13 are potential therapeutic targets for breast cancer.[96,97]

Yang et al[98] designed compound 4 based on previously reported dual CDK12/13 inhibitors, selected thalidomide and lenalidomide as E3 ligase ligands, and designed and characterized a series of CDK12/13 PROTACs by varying the length and composition of the linker chain. Evaluating the degradation efficiencies of different compounds against CDK12 and CDK13 Compound 7f was found to be highly selective for the degradation of CDK12 and CDK13. In vitro evaluation of the triple negative breast cancer cell lines MFM223 and MDA-MB-231 further demonstrated that Compound 7f almost completely degraded CDK12 and CDK13 proteins in these cells and significantly inhibited the growth and proliferation of MFM223 and MDA-MB-436 cells. 7f in combination with DDR inhibitors, such as cisplatin, showed a significant synergistic effect in inhibiting the proliferation of MDA-MB-231 cells. In conclusion, compound 7f can be used as

a valuable chemical probe to further evaluate the therapeutic potential of targeting CDK12/13 in TNBC. Thus, degradation of CDK12/13 through the use of PROTAC technology provides a new targeted therapeutic opportunity for triple-negative breast cancer.

The idea of PROTAC first appeared in 1999.[99] However, the first PROTAC did not appear until 2001.[100] Methionine aminopeptidase type 2 (MetAP-2) was the first protein to be degraded by a PROTAC, and opened the chapter of PROTACs, but the first PROTACs had low activity in human cells.[39,101] It was not until 2008 that the first small-molecule PROTAC was reported by Prof. Crews' team at Yale University, who designed a small-molecule PROTAC based on the E3 ligase MDM2 for degradation of the androgen receptor (AR), which was a major milestone in the field.[102] In 2019, the oral small-molecule targeting the AR developed by Arvinas, ARV-110 became the first PROTAC to enter clinical trials, marking an important step in moving PROTAC technology from the laboratory to clinical application.[103] In recent years, the development of small molecule inhibitors has received great attention in the field of drug development.[104] However, unlike the "placeholder-driven" mechanism of small-molecule inhibitors, the "event-driven" mechanism of PROTAC allows for lower dosage, dosing frequency, and toxicity than that of small-molecule inhibitors.[105,106] PROTAC's unique mechanism of action allows it to degrade target proteins without the need to be present at high concentrations for a long period of time, ie, a very small concentration of PROTAC is sufficient to degrade a target protein. Moreover, since it can be recycled after degrading a target protein, it is not necessary to continuously add the drug for a long time to maintain the drug efficacy. It is also possible to maintain the efficacy of the drug without the need for long-term local high concentration, the toxic side effects on the human body are much smaller, and the resistance of tumor cells to the drug will be greatly weakened. Finally, by inducing protein degradation rather than directly inhibiting its activity, the scope of use of the drug has been greatly broadened. It is expected that more drugs will enter clinical trials in the future, injecting new energy into the development of PROTAC.[107]

Conclusion and Outlook

CDKs play critical roles in cell proliferation, gene transcription and control of cell cycle progression, and they form a system to regulate the cell cycle. Ongoing research into the role of cell cycle dysregulation in BC has led to their emergence as attractive targets for cancer therapy. Similarly, Cyclins and CDKs play crucial roles in development, differentiation, and immune cell activation.[108] Immunotherapy is harnessing the body's immune system to recognize, target, and eliminate cancer cells.[109] Several types of immunotherapy strategies including Immune checkpoint inhibitors,[110] Monoclonal antibodies,[111] Antibody-drug conjugates[112] and cancer vaccines[113] have shown promising results in treating various cancers, but its drug resistance is a major limitation. At the same time, the tumor micro-environment (TME) is vital in modulating the immunotherapy response. Various tumor microenvironmental components, such as cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs), are involved in TME modulation to cause immunotherapy resistance.[114] For example, TAMs, CAFs, NK cells, T cells, lymphocytes, and other cells present in the tumor microenvironment modulate each other by secreting different cytokines and chemokines. This promotes extracellular matrix remodeling and angiogenesis and causes immune suppression in the breast cancer microenvironment.[115] The intricate interactions between the cancer cells and the immune microenvironment affect immunotherapy and many other anticancer therapies. Accordingly, there is a pressing need to ascertain novel targets and biomarkers.[116] However, targeting CDK can affect the immune microenvironment and promote anti-tumor immunity, which is promising to prevent or counteract drug resistance mechanisms.[117]

PROTAC utilizes the ubiquitin-proteasome system for targeted protein degradation of intracellular proteins to enhance the activity of existing drugs, mitigate drug side effects, and circumvent drug resistance. By designing PROTAC to degrade CDKs to better inhibit the growth of human breast cancer cells in vitro and breast cancer xenograft breast tumors, it provides a new idea for the treatment of breast cancer. However, the design of PROTAC to degrade CDKs is still in the basic research stage, and it has great potential in cancer therapy while facing significant challenges. Further clinical studies are urgently needed to validate the therapeutic potential of PROTACs, aiming to achieve precise degradation of target proteins and provide innovative therapeutic strategies for breast cancer. And could validate these findings in patient-derived xenograft models in future, so as to provide models closer to clinical reality for tumor research, drug development and precision medicine, and bring more treatment options and better prognosis for tumor patients.

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