Review Article

A narrative review of proteolytic targeting chimeras (PROTACs): future perspective for prostate cancer therapy

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Abstract: Proteolysis-TArgeting Chimeras (PROTACs) technology, as a strategy to chemically knock down transcription factors at the protein levels, can hijack the ubiquitin-proteasome degradation system to initiate the intracellular ubiquitin-proteasome hydrolysis process to degrade proteins. In the past, the development of drugs that target transcription factors has been greatly restricted, and even historically transcription factors have been regarded as "undruggable targets". PROTAC technology breaks through this limitation with its unique targeting design. With several generations of technical innovation, PROTACs have become more mature and continue to make breakthroughs in the field of targeted therapy including prostate cancer (PCa), with a new strategy for the development of anti-tumor targeted drugs. PROTACs have all the advantages of existing small molecule inhibitors, are easy to administer orally, have good cell permeability, and have wider targeting profiles compared to conventional inhibitors. The disadvantage of PROTACs is the noncancer specificity, off-target and sustained-release control, due to its catalytic role. Some androgen receptor (AR) and CDK4/6 degraders have advanced the field of PCa treatment, which is being further modified given the effects of these degraders in preclinical and clinical studies. This review summarizes in detail the technological progress and challenges that have been faced with PROTACs, the progress of research on PCa, and the prospective future of PROTACs development.

Keywords: Proteolytic targeting chimera; prostate cancer (PCa); transcription factor

Introduction

Transcription factors are a class of proteins that can bind to specific DNA sequences and are known to be highly active in humans. They can form complexes either alone or with other proteins, and play a pivotal role in regulating specific gene expression (1).

In tumors, a variety of direct or indirect mechanisms deregulate both the activity and content of transcription factors, and dysregulation of transcription factors has also become a hallmark characteristic of the tumor (2,3). Changes in chromatin structure, amplification/deletion of genes encoding the transcription factors, and mutations

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directly affect the activity of transcription factors; changes in non-coding sites, transcription factors and abnormal distribution may also indirectly affect the activity of transcription factors. However, targeting chemotherapeutic strategies has been greatly restricted, and even historically, transcription factors have been regarded as "undruggable targets". Unlike kinase proteins, kinases often have intracellular active sites that are easier to predict and recognize. Transcription factors usually work through protein-DNA or protein-protein interactions. For protein-DNA interactions, the DNA binding interface offers a highly positive charge and a convex structure, which is not conducive to target. For protein-protein interactions, the surface of the binding interface is usually flat, and the absence of a pocket structure such as a kinase active site also makes this drug development a great challenge (4,5). Despite these challenges, several generations of scientists have developed several methods to target transcription factors, including RNA interfering (RNAi), targeting of post-translational modification and degrading transcription factors with PROTACs, and targeting of intrinsically disordered regions of transcription factors and targeting the auto-inhibited state of transcription factors (6-10). The development of these new targeted drugs will greatly advance the treatment of future tumors and offer hope to patients.

In this review, we review the current knowledge regarding the PROTAC technology, the research process and the agents currently available for the treatment of PCa, and future directions for the development of the PROTAC technology. A comprehensive literature search was conducted in the PubMed/Medline, Cochrane, Scopus and ClinicalTrials databases using the keywords "prostate cancer" OR "prostate carcinoma" OR "castration-resistant prostate cancer" AND "PROTACs" OR "proteolytic targeting chimera" OR "PROTAC". We chose to include the most relevant reports based on the quality, applicability, and development of the research. We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/tau-20-1357).

Background

PROTAC (PROteolysis-TArgeting Chimera, PROTAC) is a special proteolytic targeted chimera technology (11).

PROTAC is a strategy to chemically knockdown transcription factors at the protein level. One end of the chimera contains a specific binding ligand for protein degradation, and the other end is a binding ligand for the E3 ubiquitin ligase, with both ends being connected by a linker (12).

Ubiquitin-mediated protein degradation is a critical pathway by which cells regulate their cellular protein levels. The ubiquitin-mediated protein degradation pathway can degrade more than 80% of the ubiquitous proteins in cells. This pathway plays a role in almost all cell life processes including cell cycle regulation, cell proliferation, cell apoptosis, and signaling pathways within and outside the cell (13). Among these enzymes, the E1 ubiquitin activating enzyme, E2 ubiquitin conjugating enzyme and E3 ubiquitin ligase co-operate with each other to label the substrate protein for ubiquitination, and then recruit the proteasome for degradation (14).The E3 ubiquitin ligase has clear specificity for the labeling process of substrate proteins, but also provides a theoretical and applied basis for the targeted labeling degradation of PROTACs. The special chimera structure of PROTACs narrows the space between the target protein and the intracellular E3 ubiquitin ligase by self-folding to form a target protein-PROTACs chimera-E3 ubiquitin ligase terpolymer, which makes the E3 ubiquitin ligase ubiquitinate the target protein, and then uses the ubiquitin-proteasome degradation pathway to initiate the intracellular ubiquitinating hydrolysis process to complete the process of chemically targeted degradation of the protein (15).

Research progress of proteolytic targeted chimera technology

In 2001, the research group of Professors Deshaies and Crews first reported the chemical degradation of proteins (11). The intracellular ubiquitination and degradation of specific proteins was subsequently significantly expanded and utilized. As one of the first generation of PROTACs, PROTAC-1 is one of the first PROTAC molecules. This is the first generation of peptide-based PROTACs technology, which relies on the application of comprehensive analysis of substrate binding sites and structural information for the identification of the proteins. One end of the PROTAC-1 molecule utilizes ovalicin (OVA) to cooperate with the histidine at the 231st active site of the

amino peptidase-2 (MetAP-2) peptide. The other end of the phospho-peptide of the nuclear factor κB inhibitor α (NF-κB inhibitor α, IκBα) structure can bind to β-TRCP in the E3 ubiquitin ligase protein complex. PROTAC-1 binds the MetAP-2 protein and β-TRCP complex tightly together, activating the ubiquitin-proteasome degradation pathway to degrade the MetAP-2 protein. Several subsequent peptide-based PROTACs, such as PROTAC-2, which uses the androgen receptor (AR) ligand dihydrotestosterone to link it with the IκBα phospho-peptide to degrade the AR. PROTAC-3 uses the estrogen receptor ligand estradiol and IκBα phospho-peptide to degrade the estrogen receptor (16). In addition, the larger molecular weight of the E3 ubiquitin ligand results in unstable structure and poor cell permeability. Scientists continue to discover and synthesize small molecule ligands for E3 ligases and their corresponding derivative structures, such as CRBN, cIAP, VHL and other specific E3 ligase small molecule ligands (17-20). In view of the fact that the first-generation PROTACs needs to be highly dependent on the complex use of the target protein substrate site and structure information, the second generation PROTACs based on small molecule has designed. This improvement allows PROTACs to achieve targeted degradation of common oncogenic genes and transcription factors in a variety of tumors, such as the BCR-ABL fusion protein in chronic myeloid leukemia, and the BRD4 protein in acute myeloid leukemia and lymphoma, the estrogen receptor protein in breast cancer, the TACC3 protein in fibrosarcoma, etc. (21-23).

Researchers have also found the problems of off-target and sustained-release control of tissue distribution of these small molecule PROTACs. In response to this, a research group developed the third generation PROTACs in 2013. This generation of PROTACs can specifically control the temporal and spatial distribution of PROTACs in the tissues to better exert their efficacy, and to reduce off-target distribution in the tissues. PhosphoPROTACs, known as phosphate-dependent PROTACs, use activated phosphokinase as a target signal to achieve controllable degradation (24). There are also PROTACs that use ultraviolet light sources in photodynamic therapy to achieve controllable PROTACs that can be light-controlled on and off (25).

The third-generation controllable PROTACs have become a spot for drug research. PROTACs are typically a chimera in molecular structure, with the linker connecting the two ends of the structure. The choice of a linker, the length of the linker, and the binding site between the linker and the molecules at both ends also have a greater impact on the activity of PROTACs (26). Taking estrogen receptor-targeted PROTACs as an example, when the linker causes a certain gap between the target protein binding site and the E3 ubiquitin ligase, PROTACs with 16 atom chains of the linker have the highest degradation effects (27). Drugs with PROTACs as the universal design principle are becoming increasingly common, and it is believed that controllable drugs for PROTACs will become a reality in the coming decades (28,29).

Advantages and disadvantages of PROTACs

PROTAC has gained many advantages since its inception, particularly in its unique targeting and chemical degradation. The site-directed targeting of PROTACs does not rely on directly inhibiting the active site of the kinase or inhibiting the interaction site for the purpose of inhibiting protein function (30). When designing targeted sites, there is no need to specifically analyze the active site and interaction site of the protein, nor do we consider the spatial folding structure of the site as a whole. In theory, as long as a functional site is not involved, this approach can be used to design PROTACs.

The chemical degradation of PROTACs involves the cell's own ubiquitin-proteasome protein degradation pathway. This degradation causes the protein to "disappear" within the cell after ubiquitination and hydrolysis, as opposed to specifically targeting small molecule compounds that block the active sites. This chemical degradation can minimize the shortcomings of the short half-life of small molecule compounds, and a smaller dose of PROTACs can produce significant degradation effects. Since the protein has to be re-synthesized after targeted degradation to restore its distribution and function, the effect of this chemical degradation should be more durable and efficient than small-molecule compounds that block its active site. Winter *et al.* compared the therapeutic effects of BRD4 degraders with that of BRD4 inhibitors alone. By using BRD4 inhibitors alone, BRD4 degraders can more efficiently and selectively induce protein degradation *in vitro* and *in vivo*, as well as delay the progression of leukemia in mice (31). With the continual discovery of small molecule

ligands for E3 ubiquitin ligase, the range of possibilities for the development of PROTACs has been infinitely broadened (32).

However, PROTACs are imperfect, and many potential problems have been discovered in clinical applications. Some PROTACs have poor pharmacokinetics due to their large molecular weight and cannot be easily absorbed by oral administration. The latter small molecule PROTACs improved this shortcoming and improved the oral bioavailability. Although several E3 ubiquitin ligases have been discovered, the types of small molecule ligands that can be properly matched and designed are still limited. The functioning of PROTACs is heavily dependent on the functional E3 ubiquitin ligase and the ubiquitin-proteasome protein degradation system. Once the degradation pathway fails or the E3 ubiquitin ligase mutates, drug adaptation or acquired resistance may occur. Therefore, predictive biomarkers need to be incorporated into the clinical treatment practice of PROTACs to address these issues (33).

The existing small-molecule PROTACs should have a small molecular weight after improvement, and they can enter and exit a large number of tissue cells relatively freely in the human body, resulting in a wide tissue distribution, causing serious toxic side effects. The third-generation PROTACs are a more practical strategy, but their application is limited due to the potential DNA damage and poor penetration of ultraviolet rays such as UVA. Therefore, it is possible to consider developing other light sources with better penetrability to precisely control the release of PROTACs to expand the scope of applications and reduce side effects (34).

Application of proteolytic targeting chimera technology in prostate cancer

In PCa, the androgen receptor (AR) is a key protein molecule and transcription factor closely related to tumorigenesis and development, and is also a drug target for various treatment methods including androgen-deprivation therapy (35,36). Unfortunately, most patients receiving endocrine therapy will enter the castration-resistant prostate cancer (CRPC) stage within one to two years, progress to a difficult malignant stage and eventually die from the disease (37,38). For CRPC patients, the advent of AR antagonist drugs such as enzalutamide has a significant clinical benefit for these patients (39-41). However, even

after receiving these next-generation AR antagonist drugs, patients continue to become resistant and incurable (42-44). This suggests that the still-active AR signaling pathway urgently needs to be settled.

As early as 2015, Professor Crews designed a selective androgen receptor degrader (SARD; no.SARD279). This small molecule contains a hydrophobic group linked to an AR ligand, which can induce degradation of AR proteins, reduce the expression of AR target genes and inhibit the proliferation of androgen-dependent PCa cells (45). This innovative degradation common can overcome certain resistance mechanisms that are common to traditional drugs. In 2018, Professor Crews' research group continued to expand on this technique by combining it with PROTAC technology to design a more efficient AR degradation agent, named ARCC-4 (46). ARCC-4 is a low-molecular-weight AR degradation agent that can degrade about 95% of the AR protein in PCa cells. It can significantly inhibit the proliferation of prostate tumor cells (VCaP, LNCaP, PC3, etc.), and also degrade some clinical -related AR point mutants (AR-T877A, AR-H874Y, AR-F876L, AR-L702H, AR-M896V and other point mutants). Unlike enzalutamide, ARCC-4 also has a significant anti-proliferative effect under high androgen concentration conditions, which suggests that using this degradation technology to overcome enzalutamide resistance may be a breakthrough treatment for these patients.

At the same time, a research group led by Professor Wang from the University of Michigan designed a highly effective AR-targeted PROTAC degradation agent (ARD-69) (47). *In vitro* experiments have shown that ARD-69 induces AR protein degradation in AR-positive PCa cell lines (LNCaP, VCaP and 22Rv1) in a dose- and time-dependent manner. At the same time, ARD-69 also reduces the endogenous AR protein in these PCa cell lines to below 95%, and effectively inhibits the expression of downstream regulatory genes in the AR signaling pathway. ARD-69 can effectively inhibit cell growth and has an effect that is more than 100 times that of the AR antagonist enzalutamide. In vivo experiments have shown that a single dose of ARD-69 can effectively reduce the levels of AR protein in mouse xenograft tumor tissue. These data suggest that AR-targeted degradation therapy based on PROTACs may become a more viable option for AR-positive CRPC patients. Professor Chinnaiyan's research group has also developed a new AR degrader (AR PROTAC degrader, no. ARD-61) based on

Table 1 The basic chemical structures, biological activities, physiochemical properties of the compounds. AR, androgen receptor; VHL, Von Hippel-Lindau; CDK4/6, cyclin dependent kinase 4/6

Name	Target	E3 ligase	Molecular weight (g/mol)	Molecular formula	Degradation activity
ARCC-4	AR	VHL	1,024.2	C53H56F3N7O7S2	Degradation of AR protein in VCaP cells after 20 h treatment
ARD69	AR	VHL	1,129.8	C62H74ClFN8O7S	Degradation of AR protein in LNCaP/ VCaP/22Rv1 cells after 24 h treatment
ARD61	AR	VHL	1,095.8	C61H71ClN8O7S	Degradation of AR protein in LNCaP/VCaP cells after 6 h treatment
CST620	CDK4/6	VHL	1,064.5	C55H73N11O9S	Degradation of CDK4/6 in several leukemia, myeloma and breast cancer cell lines

PROTACs and has carefully elucidated its mechanism of action. ARD-61 overcomes the existing resistance process to anti-hormone therapies by directly depleting the AR protein in both PCa and breast cancer cell lines with AR-positive features (48-50). Through *in vivo* and *in vitro* experiments, studies have found that compared to enzalutamide, ARD-61 has stronger anti-proliferation and pro-apoptosis effects, and significantly inhibits the regulation of the AR signaling in cancer cells, while still exerting an inhibitory effect in the enzalutamide resistance model. Further analysis found that although ARD-61 cannot bind and target AR-V7 (AR splice variant 7), it can still inhibit the growth of tumor cells with AR-V7 overexpression. Studies have shown that AR is still the main driving factor for PCa, and new AR degraders have a wider clinical significance for patients who are resistant to AR antagonists. The Arvinas company has been approved by the US Food and Drug Administration in 2019 to initiate a phase I clinical trial (NCT03888612) of oral PROTACs (ARV-110) targeting the AR protein in patients with metastatic CRPC. Another oral PROTAC (ARV-471) targeting estrogen receptor protein has also been approved for the Phase I clinical trial (NCT04072952) in patients with ER positive/HER2 negative locally advanced or metastatic breast cancer.

The mammalian cyclin-dependent kinases (CDKs) contain a cell cycle related sub-family (CDK1, CDK2, CDK4, CDK6) (51,52). In clinics, CDK4/6 inhibitors have emerged as a powerful class of agents for estrogen receptor positive breast cancer treatment (53). Targeting the cell cycle represents a core attack on a defining feature of cancer, given the effects of AR signaling on the cell cycle in PCa. This is a key component of the treatment of cancer. Given the importance of CDK4 and CDK6, we propose that CDK4/6 inhibitors and novel strategic combinatorial

therapies have the potential to improve patients' overall survival and quality of life (54). Steinebach *et al.* designed a VHL-based PROTAC (CST620) exhibiting dual activity against CDK4 and CDK6, and showed potent and long-lasting degrading activity in human and mouse cells and inhibited proliferation of several leukemia, myeloma and breast cancer cell lines (55). This attractive approach for targeted degradation of CDK4/6 may be further tested in PCa.

In summary, the beginning of clinical trials of oral PROTAC targeting transcription factors ends the period in which transcription factors are "undrugable". The development of these novel and exciting clinical trials provides great opportunities for future clinical applications. The basic properties of the compounds described above are listed in *Table 1* and are depicted in *Figure 1*.

Future directions

PROTACs have almost all the advantages of existing small molecule inhibitors, are easy to administer orally, have good cell permeability and solubility, and have extensive targeting profiles. As a transcription factor, the AR protein in PCa only needs to be targeted by PROTACs and does not need to consider whether it can block its active site or interfere with its interaction with other proteins, making it easier to develop and synthesize (56,57). With the progression of PCa, accumulating studies have found that the AR signaling pathway is inactivated in highly malignant neuroendocrine prostate cancer, which suggests that we need to develop appropriate drugs for patients with neuroendocrine prostate cancer. PROTACs are believed to be a progressive solution to this problem and have become an important tool in cancer treatment (58).
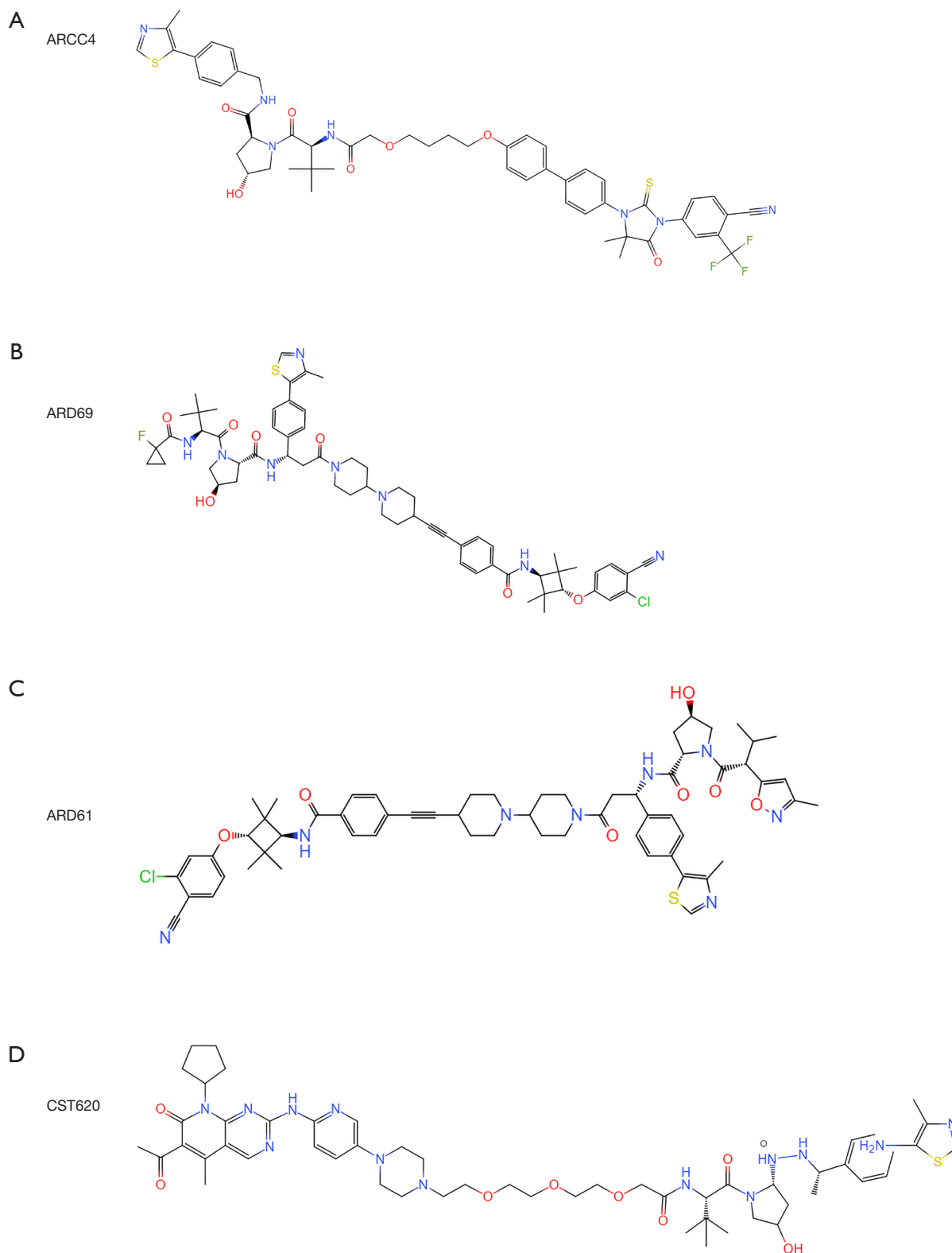
Figure 1 Chemical structures of the reported degraders. (A) Chemical structures of ARCC-4. (B) Chemical structures of ARD69. (C) Chemical structures of ARD61. (D) Chemical structures of CST60.

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Footnote

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