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PROTAC Pharmaceutical Research and its Applications

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Abstract: At present, there are very few protein targets that can be used as drugs, with approximately 15% of proteins being regulated by small molecules and biomacromolecules, while 85% of proteins currently do not have drug targets and cannot be regulated by small molecules and biomacromolecules. Proteolytic targeting chimeras (protacs) can degrade many non-pharmacological target proteins through proteasomes, thereby regulating their regulatory effects. Protein degradation targeting chimeras is an emerging direction in drug research and development. Traditional inhibitors have a blocking effect, leading to drug resistance and off target phenomena. However, this technology degrades the target protein through a 26S protease system. Structurally, PROTAC consists of three parts: the left end is a ligand that binds to the target protein, the right end is a ligand that connects to ubiquitin ligase, and the middle is connected through a "Linker". In the patient's body, one end of PROTAC is connected to E3 ubiquitin ligase, and the other end is connected to the target protein to be degraded. Through multiple rounds of ubiquitination, a ubiquitin chain is formed, achieving the UPS system to degrade the target protein. PROTAC small molecules have great prospects in the treatment of diseases. Unlike traditional small molecule drugs, they do not destroy the function of proteins, but completely degrade them. And PROTAC can be recycled, and the proteins of interest are polyubiquitinated and degraded through proteasomes. The dissociated PROTAC can also initiate new degradation, which is an important breakthrough in the fight against cancer cells. For example, we can design a PROTAC to hijack the protein required for unlimited proliferation of cancer cells, so that it can be degraded and inhibit the growth of cancer cells. Further exploration is needed for the development and clinical application of PROTAC drugs.

Keywords: PROTAC; Targeted Degradation; CRBN; VHL.

1. Introduction

1.1. Structure and Mechanism of Protein Degradation Targeted Chimera (PROTAC)

UPS is the main site for intracellular protein degradation and amino acid recycling, and this degradation process relies on the sequential action of three enzymes, E1, E2, and E3[1]. Firstly, E1 activating enzyme activates ubiquitin through an ATP dependent mechanism and generates E1 ubiquitin conjugates. Then, the E1 ubiquitin conjugate undergoes a thiol transfer reaction with the E2 binding enzyme to generate the E2 ubiquitin conjugate [2]. Finally, E2 ubiquitin conjugates transfer ubiquitin to POI mediated by E3 ligase, ubiquitination of POI, resulting in POI degradation by proteasomes. PROTAC technology is a chemical knockout strategy inspired by this biological process. Its core mechanism is to use PROTAC molecules to form a ternary complex (POI · PROTAC · E3) with POI and E3 ligases, thereby making POI ubiquitinated and subsequently degraded by UPS [3] [4].

PROTAC molecules generally do not undergo degradation during this process, so they can be reused to achieve sustained ubiquitination and degradation of POI [5]. In PROTAC technology, suitable PROTAC molecules are crucial. To bind to both POI and E3 ligases simultaneously, the PROTAC molecule requires three functional units, namely POI binding ligand, E3 ligase binding ligand, and linker. Under appropriate conditions, these three functional units are relatively independent, so they can be designed and optimized separately [6]. Components with different structures but the same function can also be replaced with each other. Therefore, PROTAC molecules have the characteristic of "plug and play" and are very convenient to use [7].

At present, PROTAC molecules are mainly composed of chemical small molecules, and the binding ligands of POI and E3 ligases include two types: covalent and non-covalent small molecules. The binding ligands of POI and E3 ligase can be selected separately, sometimes by means of virtual screening. When selecting POI binding ligands, it is required that the molecules have high POI specificity, but not necessarily a high affinity between the two. When selecting E3 ligands for binding to ligands, molecules should have good physical and chemical properties, usually with high affinity, selectivity, and cellular activity, in order to efficiently and specifically recruit E3 ligases and improve the efficacy of PROTAC [8][9]. After obtaining POI and E3 ligase binding ligands, it is necessary to introduce modifying groups and add linkers at positions that do not affect the binding function of these two parts, and assemble them into complete PROTAC molecules. As the junction structure between POI binding ligand and E3 ligase binding ligand, the linker has an important influence on the stability and subsequent functions of ROTAC and POI PROTAC E3 ternary complex. In order to maintain the delicate balance between PROTAC affinity and spatial effect, the linker should have an appropriate stereochemistry structure [10]. The results showed that the activity, selectivity and physicochemical properties of PROTAC were directly affected by the type and length of linkers. At present, the commonly used linkers mainly include four categories: alkyl, ether, amino acid, and polyethylene glycol l [11].

2. Characteristics of Drugs based on PROTAC Technology

The development of traditional protein small molecule inhibitors often faces many challenges: 1) some proteins lack active binding pockets that directly regulate protein function; 2) High concentration drug exposure to achieve the

occupation of active site will lead to potential risk of off target toxicity; 3) The selectivity of small molecule inhibitors targeting the active pocket sites of kinase proteins is low; 4) Easy to develop drug resistance. At the same time, although macromolecular drugs have advantages such as strong specificity, low dosage and frequency of administration, they generally have problems such as high production costs, inconvenient delivery methods, mainly targeting membrane proteins, and difficulty in penetrating the blood-brain barrier. Compared to traditional "occupancy driven" small molecule drugs and large molecule drugs, PROTAC has significant advantages, mainly including the following aspects [12][13].

2.1. Transforming Impossible Drug Targets into Feasible Drug Targets

Traditional small molecule drugs directly bind to the active pocket of the target protein to exert pharmacological activity. However, some proteins have relatively smooth surfaces, lack obvious binding "pockets", lack hydrogen bond donors and receptors, and are difficult to develop targeted inhibitors, known as "non producible" proteins [14]. According to statistics, up to 80% of the disease related proteins that have been analyzed are difficult to achieve drug targeted inhibition. Some of these proteins are located within cells or nuclei, and macromolecular drugs cannot reach the target protein site. Unlike "occupancy driven" small molecule drugs and macromolecular drugs, "event driven" targeted protein degradation agents do not need to act on the active site of the protein to inhibit its activity, expanding the scope of "drug ready" targets, including those without enzyme activity or signal function, such as scaffold proteins, non-functional protein aggregates, transcription factors, etc. [15][16].

2.1.1. Can Improve Selectivity, Activity, and Safety

The traditional pharmacological action mode of small molecule inhibitors is the "occupancy driven" mode. In order to improve target occupancy, high-dose drugs are often required, which can bring potential off target toxicity. PROTAC has a catalytic degradation effect, and its EC50 value to cells is much lower than the intrinsic target binding Kd value, indicating that a low level of occupation of the target protein is sufficient to maintain the degradation rate of the target protein, while maintaining good activity and improving safety. At the same time, the degradation induced by PROTAC is usually sustained, and PROTAC can degrade the target protein to a lower level within a few hours. After PROTAC is completely cleared, cells may still need a considerable period of time to restore the protein to its normal physiological level, greatly extending the duration of PROTAC's action. It was found that PROTAC based on multi target protein kinase inhibitors achieved target selective degradation [17]. In 2018, it was reported that based on the non-selective kinase inhibitor Foretinib, although it can bind more than 190 kinases, due to the unique protein-protein interaction between E3 ligases and degradation targets, only 12 and 22 kinases can be degraded in cell experiments, greatly improving the selectivity of the target [18].

2.2. Can Reduce Cell Resistance and have Broad-Spectrum Properties

Common target protein related resistance (on target resistance) includes overexpression of targets and secondary mutation resistance, the former leading to an increase in drug use, while the latter leads to a significant decrease in drug affinity. However, the demand for PROTAC molecule binding ligands has weakened, which can effectively catalyze the degradation of target proteins and their mutants, thereby overcoming the resistance problem of traditional inhibitors. Telaprevir was once abandoned in clinical practice due to drug resistance issues. In 2019, researchers prepared a degradation agent DGY-08-097 targeting HCVNS3/4A with telaprevir as the target protein binding ligand. Compared to wild-type viruses, the antiviral efficacy of telaprevir against NS3-V55A is reduced to about one-third, while DGY-08-097 exhibits similar antiviral activity in wild-type viruses and NS3-V55A mutant viruses [19].

3. Summary

IIt has been more than 20 years since the concept of PROTAC was proposed and PROTAC drugs entered clinical evaluation. As an emerging technology, PROTAC has attracted great attention from both academia and the pharmaceutical industry. Especially in the past few years, PROTAC molecules have shown astonishing results in phase I and phase II clinical trials. At present, PROTAC has been used for the degradation of over 80 targets and over 100 proteins, with in-depth research and outstanding achievements in the treatment of tumors, viral infections, and neurodegenerative diseases, becoming a hot and important direction for the development of new generation drugs.

E3 ligase is a key component of proteasome mediated protein degradation. There are over 600 known E3 ligases in the human body, but so far, less than 1% of E3 ligases have reported small molecule ligands, and over 90% of reported PROTACs are recruited through a few E3 ligases such as CRBN, VHL, MDM2, cIAP, etc. Although the development of PROTAC based on VHL and CRBN is becoming increasingly mature, there are still challenges in its preclinical and clinical development. Research has found that mutations or downregulation of the components of ubiquitin ligases can lead to the emergence of drug resistance in PROTAC degrading agents based on CRBN or VHL. Therefore, it is still necessary to develop new E3 ubiquitin ligases and their ligands. At the same time, in the future, efficient E3 ligands will be selected based on the differential expression of E3 ligase in different tissues and cells to improve the selectivity of PROTAC and provide therapeutic methods for protein degradation therapy targeting specific tissues.

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