INTRODUCTION

Gastrointestinal cancers are the most common cancers with the highest mortality globally, and are characterized by insidious early symptoms, high aggressiveness, and rapid progression. According to the statistics of the American Cancer Society, the number of projected new cases of gastrointestinal cancer in the USA was 338,090 for 2021, while the number of projected deaths was 169,280 for that year. Among them, colorectal cancers and pancreatic cancers ranked the first and third in malignancy deaths, respectively. Comprehensive tumor therapies, including surgery, endoscopic treatment, chemotherapy, radiation therapy, and targeted therapy can improve the survival rate of patients with gastrointestinal cancers. However, the prognosis of patients with gastrointestinal cancers depends mainly on the stage at the time of diagnosis, and there are still limitations in the current treatment of advanced cancers. Therefore, it is necessary to find new effective treatment strategies for patients with gastrointestinal cancers.

Although small-molecule inhibitors (SMIs) and monoclonal antibodies (mAbs) targeting aberrantly expressed or mutated proteins have been used to treat cancer for many years, most proteins without enzymatic function (such as transcription factors and scaffolding proteins) lack active sites for the binding of SMIs or mAbs and are defined as non-drug targets. Moreover, SMIs and mAbs have their own limitations. For instance, mAbs cannot pass through cell membranes. Therefore, they can only act on targets that are expressed or secreted on the cell surface. By contrast, SMIs can target both intracellular and plasma membrane proteins. The main disadvantages of SMIs are resistance, toxicity, selectivity, and off-target effects. In the last 20 years, an effective technology for endogenous protein degradation using proteolysis targeting chimera (PROTACs) has emerged. The ligands of the PROTAC target proteins active binding sites but also transcription factors, scaffolding proteins, and intracellular proteins. Thus, PROTACs can overcome the shortcomings of both SMIs and mAbs. Unlike SMIs that inhibit target proteins, PROTACs can overcome drug resistance caused by target mutations or overexpression by degrading target proteins.

Increasing evidence suggests that PROTACs can be used for drug development. For instance, AR-targeted PROTAC degraders ARCC-4, AR-D9, AR-D61, AR-D266, and WWL0245 have been shown to degrade the androgen receptor (AR) and inhibit prostate cancer cell proliferation. Other studies have found
that bromodomain-containing protein 4 (BRD4)-targeted PROTAC degraders MZ1 and ARV-825 can downregulate BRD4 protein expression levels and exhibit anti-proliferative effects in triple-negative breast and ovarian cancers. Importantly, oral AR-targeted PROTAC ARV-110 and ER-targeted PROTAC ARV-471 have shown efficacy in phase I clinical trials in prostate cancer (NCT03888612) and breast cancer (NCT04072952), respectively. Therefore, this paper expounds the mechanism and research progress of PROTAC, and summarizes its application in gastrointestinal cancers. We aim to provide new strategies for antitumor targeted therapy and develop more effective antitumor drugs for gastrointestinal cancers.

**PROTAC: A NOVEL STRATEGY FOR PROTEIN DEGRADATION**

**The mechanism of PROTAC**

PROTAC is a technique that was first proposed in 2001; it removes specific oncopgenic proteins from cells by mobilizing its own protein degradation pathway, serving as an alternative to antitumor targeted therapy. PROTACs are heterologous bifunctional molecules consisting of a POI conjugate, a linker, and an E3 ligase ligand. Their degradation function is achieved by using cell UPS. UPS is divided into the following three major categories: Really Interesting New Gene (RING), homologous to the E6AP carboxyl terminus (HECT), and RING-in-between-RING (RBR) ligases. The human genome contains more than 600 E3 ligases. They are required for the corresponding E3 ligase, and this can be ascribed to the following factors: (1) the shape complementarity between the ligase and POI, (2) the ability of ligase and target to form degradable POI-ligase complexes, (3) the subcellular localization of ligase and POI, and (4) the tissue and cell specificity of ligases and targets. Therefore, this section describes the research progress on PROTACs based on different types of E3 ligases. The main events and milestones in the development of PROTAC are shown in Figure 2.

**First generation: Peptide-based PROTACs**

The first PROTAC is PROTAC-1, which targets methionine aminopeptidase-2 (MetAP-2) ubiquitinated degradation, reported by Sakamoto et al. in 2001. PROTAC-1 mediates the degradation of MetAP-2 in unfertilized Xenopus laevis egg extracts by recruiting SCFß-TrCP E3 ligase. Two years later, Sakamoto and Deshaies’ team reported that PROTACs targeted AR and estrogen receptor (ER) for ubiquitination and degradation in prostate and breast cancers, respectively, expanding future approaches in cancer treatment. In 2004, Crews’ group reported the first PROTAC molecule with cellular permeability. Upon entry into the cell, it is recognized and hydroxylated by prolyl hydroxylase. It then binds to von Hippel-Lindau (VHL) E3 ligase and mutant FK506-binding protein (FKBP12). Finally, FKBP12 is induced to be ubiquitinated and degraded (Figure 1). This process eliminates the need for the POI ligand to occupy the binding site for long periods of time and requires only a brief formation of the ternary complex to transiently ubiquitinate POI. In this process, PROTAC can serve as a catalyst within the cell. Therefore, they exhibit excellent anti-tumor activity, usually at nanomolar concentration of half-degrading concentrations (DC50).

**Research progress on PROTACs**

The human genome contains more than 600 E3 ligases. They are divided into the following three major categories: Really Interesting New Gene (RING), homologous to the E6AP carboxyl terminus (HECT), and RING-in-between-RING (RBR) ligases. Among them, Cullin-RING ligase (CRL) is the largest family of E3 ubiquitin ligases containing a RING structure. Specific POI degradation requires the corresponding E3 ligase, and this can be ascribed to the following factors: (1) the shape complementarity between the ligase and POI, (2) the ability of ligase and target to form degradable POI-ligase complexes, (3) the subcellular localization of ligase and POI, and (4) the tissue and cell specificity of ligases and targets. Therefore, this section describes the research progress on PROTACs based on different types of E3 ligases. The main events and milestones in the development of PROTAC are shown in Figure 2.
VHL is a substrate receptor for the CRL complex CRL2 VHL. Since then, many studies have designed peptide-based PROTACs with greater cellular permeability, higher affinity, and higher degradation efficiency by modifying VHL or POI ligands. These PROTACs inhibit hormone-dependent prostate and breast cancers by targeting the ubiquitinated degradation of steroid hormone receptors. These groundbreaking studies ushered in the era of PROTAC technology. Simultaneously, modifiable PROTACs also show potential for cancer treatment.

**Second generation: Small-molecule-based PROTACs**

The first-generation peptide-based PROTACs have limitations owing to their low cell permeability and chemical stability in vivo. In this context, Crew reported the first PROTAC based on a small-molecule E3 ligase ligand in 2008. This PROTAC consists of a murine double minute 2 (MDM2) ligand called nutlin and a nonsteroidal AR ligand (SARM) connected by a polyethylene glycol (PEG)-based linker, which induces AR ubiquitination and degradation by the proteasome. Small-molecule-based PROTACs are characterized by low molecular weight, better stability and cell permeability, higher target selectivity, rapid and durable degradation efficiency, significant oncogenic effects, and defense against drug resistance caused by SMIs. Therefore, an increasing number of studies are focusing on small-molecule-based PROTACs.

The second class of E3 ligases utilized by small-molecule PROTACs is a family of anti-apoptotic proteins known as inhibitor of apoptosis (IAP). In 2010, Hashimoto et al. designed and synthesized hybrid molecules utilizing the ubiquitin E3 ligase activity of cIAP1 to degrade cellular retinoic acid-binding proteins (CRABPs) via the UPS pathway. This hybrid molecule used methyl bestatin (MeBS) as a cIAP1 ligand and all-trans-retinoic acid (ATRA) as CRABPs. Subsequently, many studies developed a series of hybrid molecules consisting of target ligands and bestatin based on IAP, called specific and nongenetic IAP-dependent protein eraser (SNIPER). They induced specific degradation of POIs (including ERz, AR, BRDs, BCR-ABL, BTK, and TACC3) by UPS.

To date, VHL and cereblon (CRBN) have been the most widely used E3 ligases for the design and synthesis of small-molecule-based PROTACs. VHL is a component of a multi-subunit E3 ligase that recognizes the hypoxia-inducible factor protein HIF1-α and labels it for proteasomal degradation. The first VHL ligand was designed and synthesized by Crews and co-workers in 2012. All VHL-based small-molecule PROTACs emerged after the discovery of the VHL ligand by the Crews’ group. In 2015, Crews’ group proposed the first VHL-based small-molecule PROTACs targeting estrogen-related receptor α (ERRα) and receptor-interacting serine/threonine protein kinase 2 (RIPK2). Since then, several research teams have designed a series of targeted protein degradation (TPD) by PROTACs based on the small-molecule VHL, which has made significant progress in the field of tumor therapy. VHL-based PROTACs can effectively degrade ERRz, RIPK2, BRD4, BCR-ABL, ALK fusion proteins (NPM-ALK and EML4-ALK), epidermal growth factor receptor (EGFR), HER2, c-Met, SGK3, and Cdc20, exhibiting potent antitumor effects.

CRBN is a component of the cullin-RING E3 ligase CUL4-RBX1-DDB1-CRBN (CRL4-CRBN). CRBN recruits zinc-finger transcription factors...
factor or kinase CK1a to mediate CUL4-CRBN-dependent ubiquitination and proteasomal degradation. Handa’s team identified CRBN as a thalidomide-binding protein. In 2015, the first CRBN-based PROTAC-dBET1 was developed; dBET1 consists of a CRBN ligand and a ligand for the BRDs inhibitor JQ1, which specifically and efficiently degrades the BET family members BRD2/3/4. They successfully developed dFKBP1, which specifically degrades the cell membrane signaling protein FKBP12. In the same year, Crews’ group synthesized ARV-825, consisting of a classic BRD4-binding moiety of the triazolo-diazepine acetamide class seen in OTX015 and pomalidomide via PEG. ARV-825 exhibited more potent inhibitory effects on c-MYC levels and downstream signaling than BRD4 inhibitors, ultimately inhibiting cell proliferation and inducing apoptosis in Burkitt’s lymphoma (BL). After this, a significant number of CRBN-based PROTACs have been designed. They are characterized by high specificity, acceptable physicochemical properties, a broad targeting spectrum, and good structural characteristics. Therefore, CRBN-based PROTACs are widely used to degrade target proteins in various diseases, including neurodegenerative and cardiovascular diseases, immune disorders, and cancers.

Despite the wide variety of E3 ligases, few are currently used for PROTACs. Therefore, the development of novel PROTACs requires the involvement of more E3 ligases. In recent years, studies have focused on E3 ligases, including DCAF5, F box protein family, fem-1 homolog B (FEM1B), and Cullin-RING protein 4/114 (RNF4/114). These are expected to make outstanding contributions to the fields of PROTAC and TPD.

### Third generation: Spatiotemporal-manner-controlled PROTACs

In most cases, drugs interact with target proteins in both target and non-target tissues. When the drug interacts with multiple non-target tissues, it exhibits an adverse effect known as the off-target tissue effect. In addition, PROTACs also have unintended effects, called drug toxicities, which limit their clinical application. Therefore, many researchers have worked on the development of spatiotemporal-manner-controlled PROTACs. We summarized spatiotemporal-manner-controlled PROTACs in Table 1, and the corresponding chemical structures in Figure 3.

Phospho-dependent PROTACs. In 2013, Crew’s team successfully designed two phosphoPROTACs. This type of PROTAC relies on phosphorylation for protein degradation. They couple the tyrosine phosphorylation sequence of the nerve growth factor tropomyosin receptor kinase A (TrkA) or the neuregulin receptor erythroblastosis oncogene B3 (ErbB3) to the VHL ligand. Therefore, they can recruit and degrade fibroblast growth factor receptor substrate 2a (FRS2a) or phosphatidylinositol 3-kinase (PI3K) after phosphorylation of PROTACs by receptor tyrosine kinase activation (Figure 4A).

Photoswitchable PROTACs. In 2019, Carreira et al. designed a photoswitchable PROTAC (photoPROTAC) with ortho-F4-azobenzene linkers. The bistable property of photoPROTAC is controlled by regulating the topological distance between the two ligands, and the photoswitchable state is persistent and reversible. The azo-cis-isomer with a short topological distance is in an inactive state, whereas the azo-trans isomer with a long topological distance is in an active state. Based on this principle, the authors designed photoPROTAC-1. Under 415-nm irradiation, trans-photoPROTAC-1 induced significant degradation of BRD2. However, under 530-nm irradiation, trans-photoPROTAC-1 was converted to inactivated cis-photoPROTAC-1. Since then, Trauner et al. used this principle to synthesize photochemically targeting chimeras (PHOTACs). They synthesized CRBN-based PHOTAC-I-3, PHOTAC-I-10, PHOTAC-II-5, and PHOTAC-II-6 by introducing

### Table 1. Third generation: spatiotemporal-manner-controlled PROTACs

| Third-generation PROTACs | PROTACs | E3 ligase | Target | Reference |
|-------------------------|---------|-----------|--------|-----------|
| Phospho-dependent PROTAC | phosphoPROTAC | VHL | FRS2α, PI3K | Hines et al. |
| PHOTAC | trans-photoPROTAC-1 | VHL | BRD2 | Pfaff et al. |
| PHOTAC | cis-photoPROTAC-1 | VHL | BRD2 | Pfaff et al. |
| PHOTAC-I-3 | CRBN | BRDs | Reynders et al. |
| PHOTAC-I-10 | CRBN | BRDs | Reynders et al. |
| PHOTAC-II-5 | CRBN | FKBP12 | Reynders et al. |
| PHOTAC-II-6 | CRBN | FKBP12 | Reynders et al. |
| Photoswitchable PROTACs | ERRα PROTAC 2 | VHL | ERRα | Naro et al. |
| PHOTAC | BRD4 PROTAC 4 | VHL | BRD4 | Naro et al. |
| PHOTAC | pc-PROTAC1 | CRBN | BRD4 | Xue et al. |
| PHOTAC | pc-PROTAC3 | CRBN | BTK | Xue et al. |
| PHOTAC | opto-dBET1 | CRBN | BRDs | Liu et al. |
| PHOTAC | opto-dALK | CRBN | ALK | Liu et al. |
| PHOTAC | SPN pro | VHL | IDO | Zhang et al. |
| PHOTAC | PhotoPROTAC N2 | CRBN | BRD4 | Li et al. |

Photoswitchable PROTACs. In 2019, Carreira et al. designed a photoswitchable PROTAC (photoPROTAC) with ortho-F4-azobenzene linkers. The bistable property of photoPROTAC is controlled by regulating the topological distance between the two ligands, and the photoswitchable state is persistent and reversible. The azo-cis-isomer with a short topological distance is in an inactive state, whereas the azo-trans isomer with a long topological distance is in an active state. Based on this principle, the authors designed photoPROTAC-1. Under 415-nm irradiation, trans-photoPROTAC-1 induced significant degradation of BRD2. However, under 530-nm irradiation, trans-photoPROTAC-1 was converted to inactivated cis-photoPROTAC-1. Since then, Trauner et al. used this principle to synthesize photochemically targeting chimeras (PHOTACs). They synthesized CRBN-based PHOTAC-I-3, PHOTAC-I-10, PHOTAC-II-5, and PHOTAC-II-6 by introducing

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an azobenzene photoswitch into the linkage region of dBET1 or dFKBP12. Under light, PHOTAC-I-3 and PHOTAC-I-10 degraded BRDs, whereas PHOTAC-II-5 and PHOTAC-II-6 degraded FKBP12 (Figure 4B).

Photocaged PROTACs. Several studies have attempted to prevent PROTAC from forming ternary complexes with E3 ligase and POI by installing a photo-unstable protective caging group on the E3 ligase ligand. Finally, photocaged PROTAC must be triggered by light to complete protein degradation (Figure 4C). Deiters’ group designed caged ERRα PROTAC 2 by installing a diethylamino coumarin (DEACM) at the hydroxyl group via a carbonate linkage and synthesized caged BRD4 PROTAC 4 by installing a 6-nitropiperonyloxymethyl (NPOM) group at the glutarimide nitrogen. Upon UV irradiation, the caged ERRα PROTAC 2 and the caged BRD4 PROTAC 4 mediated the degradation of ERRα and BRD4, respectively.70 Pan’s team installed the bulky 4,5-dimethoxy-2-nitrobenzyl (DMNB) group at the amide nitrogen of the JQ1 and the imide nitrogen of MT-802 to synthesize pc-PROTAC1 and pc-PROTAC3 for degradation of BRD4 and BTK, respectively.71 Recently, Liu et al. generated inert opto-pomalidomide by adding a nitroveratryloxy carbonyl group as the lead parental compound to the glutarimide nitrogen. This inert opto-pomalidomide can be photolyzed under UVA (UVA) irradiation and reverted to active pomalidomide, thereby inducing the ubiquitinated degradation of IKZF1/3 (Ikaros zinc finger transcription factor 1/3). Based on this principle, they developed opto-dbET1 and opto-dALK, which mediate the degradation of BRD2/3 and NPM-ALK fusion protein in a spatiotemporal manner, respectively.72 Pu’s group designed semiconducting polymer nanoparticles PROTAC (SPN pro) consisting of semiconducting polymer nanoparticles (SPNs) attached to IPCP (PROTAC peptide-IPP with histone B cleavable fragments) via PEG.73 IPP is a PROTAC peptide composed of the indoleamine 2,3-dioxygenase (IDO) targeting unit NLG919 (an IDO inhibitor) and the VHL ligand via a linker. Under near infrared (NIR) photoirradiation, cleavage of SPN pro by histone B (Cat B) releases IPP with IDO degradation activity. Another study synthesized photoPROTAC N2 for photocontrol of BRD4 protein degradation by linking ortho-nitrobenzyl derivatives and BRD4-PROTAC dBET1 to the same glutarimide moiety.74

Taken together, the photocontrolled generation or release of active PROTACs can control their tissue distribution and reduce off-target effects to improve efficacy. More importantly, these latest studies on spatiotemporal-manner-controlled third-generation PROTACs provide a practical approach for their future clinical application.

PROTACs in clinical trials
PROTACs have been increasingly studied in cancer treatments. However, their safety, toxicity, and therapeutic effects in humans are still elusive.37 It was not until ARV-110 and ARV-471 were applied to phase I clinical trials in prostate cancers and breast cancers, respectively, that the avenue of PROTAC in clinical application

Figure 3. Chemical structure of third-generation PROTACs
was opened.25 Preliminary data from a phase I clinical trial of oral ARV-110 reported favorable safety and tolerability of ARV-110 in patients with metastatic desmoid-resistant prostate cancer (mCRPC). The data showed that ARV-110 reduced prostate-specific antigen (PSA) levels in patients, which also revealed its antitumor activity. However, patients treated with the combination of ARV-110 and rosuvastatin showed significant AST/ALT elevations followed by renal failure.13 This suggests that the combination of ARV-110 and rosuvastatin can cause serious toxic side effects. Therefore, the combined use of ARV-110 and rosuvastatin should be avoided in future clinical applications.13,37 The initial efficacy of ARV-110 in treating mCRPC led to its entry into a phase II clinical trial (NCT03888612). Preliminary data from the phase I clinical trial of ARV-471 showed that it significantly degraded wild-type and mutant ER in breast cancer patients. Furthermore, the Response Evaluation Criteria in Solid Tumors (RECIST) evaluation revealed that some breast cancer patients treated with ARV-471 achieved stable disease or even partial remission. In addition, ARV-471 showed good tolerability and safety with no grade 3 or higher adverse reactions in treatment-related adverse events.13,37 ARV-471 monotherapy for metastatic breast cancer has also entered a phase II clinical trial (NCT04072952). Since then, an increasing number of PROTACs have entered phase I clinical studies, such as ARV-766 (NCT05067140) and CC-94676 (NCT04428788) targeting AR, AC682 (NCT05080842) targeting ER, DT2261 (NCT04886622) targeting BCL-xL, FHD-609 (NCT04965753) and CFT8634 (NCT05355753) targeting BRD9, KT-474 (NCT04772885) and KT-413 (NCT05233033) targeting IRAK4, NX-2127 (NCT04830137) and NX-5948 (NCT05130122) targeting BTK, CFT7455 (NCT04756726) targeting IKZF1/3, and KT-333 (NCT05225584) targeting STAT3.

APPLICATION OF PROTEOLYTIC TARGETING CHIMERAS IN GASTROINTESTINAL CANCER

Gastrointestinal cancers include esophageal, stomach, liver, bile duct, gallbladder, pancreas, small intestine, and colorectal cancer. Most patients with gastrointestinal cancer have insidious early symptoms and are mostly at an advanced stage at the time of diagnosis. As a result, treatment options are limited and patient prognosis is often poor. PROTAC, an emerging technique, has shown promising efficacy in phase I clinical trials for prostate and breast cancer. This has inspired researchers to explore the mechanisms and the efficacy of PROTACs in gastrointestinal cancers, especially pancreatic cancers (Figure 5) and colorectal cancers. We summarize PROTACs applied to gastrointestinal cancers in Table 2 and the corresponding chemical structures in Figure 6.

Application of PROTACs in esophageal carcinoma

Src homology 2 domain-containing phosphatase 2 (SHP2), a protein tyrosine phosphatase (PTP), was initially found to be associated with neurotrophin signaling.101,102 Activating mutations in SHP2 have been found to be involved in multiple oncogenic signaling pathways in a various hematologic and solid tumors.103-107 Therefore, SHP2...
can serve as a key target for cancer therapy. SHP099 is a potent metamorphic SHP2 inhibitor that selectively inhibits the phosphatase activity of SHP2, thereby inhibiting cancer cells and tumor growth.\textsuperscript{108,109} SHP099 Wang’s team designed a PROTAC with SHP099-derived SHP2 inhibitor and VHL ligand that can efficiently degrade SHP2 and named it SHP2-D26. SHP2-D26 degraded SHP2 protein in the esophageal carcinoma KYSE520 cell line and human acute monocytic leukemia (AML) MV4;11 cell line in a time- and dose-dependent manner. In addition, their study showed that SHP2-D26 was superior to the SHP2 inhibitor SHP099 in dose-dependent inhibition of extracellular signal-regulated kinase (ERK) phosphorylation and cell proliferation. In summary, SHP2-D26 is the first potential PROTAC degrader of SHP2 protein and may have good applications in solid and hematological tumors.\textsuperscript{86}

Application of PROTACs in gastric cancer

It has been shown that the BET protein family inhibitor JQ1 can inhibit cell proliferation and migration in gastric cancer.\textsuperscript{110,111} Cui’s group found that BRD4 mRNA was highly expressed in gastric cancer tissues and was significantly associated with poor prognosis. Therefore, targeting BRD can be an important strategy for gastric cancer treatment. ARV-825 is a heterogeneous bifunctional PROTAC molecule that efficiently degrades BRD4/2/3. It consists of the BET protein inhibitor (BETi) OTX015 and pomalidomide targeting the CRBN E3 ligase connected by an ethoxy spacer group.\textsuperscript{59} ARV-825 inhibits cell proliferation and migration, and induces apoptosis and cell cycle arrest in gastric cancer cells \textit{in vitro}. In addition, ARV-825 was shown to have stronger antitumor effects than the BET protein family inhibitors OTX015 and JQ1. The downstream signaling molecules of the BET protein family included c-Myc, Polo-like kinase 1 (PLK1), cystathione 3, and poly-ADP-ribose polymerase (PARP). They were downregulated with the degradation of BRDs. Ultimately, ARV-825 inhibited the growth of gastric cancer cells and promoted apoptosis.\textsuperscript{87}

Applications of PROTACs in hepatocarcinoma

TD-165 is a novel PROTAC that degrades CRBN. Depletion of CRBN by TD-165 increased the expression level of the L-type voltage-dependent Ca\textsuperscript{2+} channel (LTCC) isoform Cav1.2z and LTCC current (IC\textsubscript{L}), ultimately showing efficacy in heart failure with reduced ejection fraction (HFrEF).\textsuperscript{112} CRBN was reported to contain a conserved N-terminal structural domain of Lon protease, which was named Lon.\textsuperscript{113,114} The mitochondrial Lon protease (Lonp1) is a key regulator of mitochondrial homeostasis.\textsuperscript{115} Dysregulated expression of Lonp1
produces excess reactive oxygen species (ROS) and is involved in aging, infertility, and tumorigenesis. Thus, CRBN has a protective function in mitochondria. Another study showed that knockdown of CRBN in the hepatoblastoma HepG2 cell line and cervical cancer HeLa cell line resulted in increased ROS production, increased oxidative stress, mitochondrial calcium overload, reduction of mitochondrial membrane potential, increased cytochrome c levels, and superoxide accumulation. Therefore, mitochondria undergo irreversible damage induced by ROS, which ultimately inhibits the proliferation, viability, and cell cycle progression (manifested by an increase in G1 and G2/M phase cells, and a decrease in S phase cells) of HepG2 and HeLa cells, and induces apoptosis. In addition, the levels of apoptotic markers, including caspase3/7, cleaved PARP, cleaved cystathione 7, p53, and p21, were increased in CRBN-knockdown cells. Moreover, long-term depletion of CRBN caused by the use of TD-165 induces irreversible mitochondrial dysfunction, which eventually leads to cell death. Taken together, TD-165 may serve as a novel strategy for the treatment of hepatoblastoma and cervical cancer by targeting CRBN.

Table 2. Application of PROTACs in gastrointestinal cancer

| Cancer             | PROTACs               | E3 ligase | Target         | Reference |
|--------------------|-----------------------|-----------|----------------|-----------|
| Esophageal carcinoma | SHP2-D26             | VHL       | SHP2           | Wang et al. |
| Gastric cancer     | ARV-825               | CRBN      | BRDs           | Liao et al. |
| Hepatocarcinoma    | TD-165               | VHL and CRBN | CRBN          | Park et al. |
|                    | BETd-260             | CRBN      | BRD4           | Zhang et al. |
|                    | PROTACx-protein      | VHL       | X protein      | Monteiro and Krissansen |
| Cholangiocarcinoma | ARV-825              | CRBN      | BRDs           | Lu et al. |
|                    | DX2-145              | CRBN      | GRP78          | Samanta et al. |
|                    | DP-C 1               | CRBN      | EGFR and PARP  | Zheng et al. |
|                    | DP-C 2               | CRBN      | EGFR and PARP  | Zheng et al. |
|                    | DP-C 3               | CRBN      | EGFR and PARP  | Zheng et al. |
|                    | DP-C 4               | CRBN      | EGFR and PARP  | Zheng et al. |
| Pancreatic cancer  | PRTC                 | VHL       | CREPT          | Ma et al. |
|                    | ARV-NP(ARV-825)      | CRBN      | BRDs           | Saraswat et al., 2020; Minko |
|                    | PROTAC(SLUG)         | CRBN      | SLUG           | Nabet et al.; Bilal et al. |
|                    | XD2-149              | CRBN      | ZQ01, ZFPP1    | Hanafe et al. |
|                    | PROTAC (CDK6)        | CRBN      | CDK6           | Rana et al. |
| Colorectal cancer  | DT2216               | VHL       | BCL-xL         | Thumman et al.; Khan et al. |
|                    | xStAxs               | VHL       | β-catenin      | Liao et al. |
|                    | A1874                | MDM2      | BRD4           | Qin et al. |
|                    | dSBT1                | CRBN      | BRD4           | Otto et al. |
|                    | MZ1                  | VHL       | BRD4           | Otto et al. |
|                    | PROTAC(CDK9)         | CRBN      | CDK9           | King et al. |
|                    | PROTAC 4             | CRBN      | HDAC9          | Small et al. |
|                    | dBET6                | CRBN      | BRD4           | Kapoor et al. |
|                    | PROTAC (PARP-1)      | CRBN      | PARP-1         | Guo et al. |
|                    | PROTAC-21a           | CRBN      | PD-L1          | Wang et al. |
|                    | PROTAC-O412          | CRBN      | SF3B1          | Gama-Brambila et al. |

BRD4 is overexpressed in most hepatocellular carcinoma (HCC) tissues, suggesting that BRD4 could be a potential therapeutic target for HCC. In addition, a novel PROTAC that can efficiently degrade BRD4 in leukemia was prepared and named BETd-260. BETd-260 consists of the BET inhibitor HJB-97 and thalidomide linked by an azacarbazole-based linker. Thus, based on the efficient degradation of BRD4 by BETd-260 in leukemia, Zhang’s team explored the activity of BETd-260 in HCC. Their study showed that BETd-260 induces ubiquitinated degradation of BRD2/3/4 in a dose-dependent manner. Subsequently, the expression of c-Myc, Mcl-1, and Bcl-2, and XIAP, the downstream anti-apoptotic genes of BRDs, was downregulated. It has been documented that the intrinsic apoptotic signaling pathway is regulated by the Bcl-2 and IAP protein families. Thus, BETd-260 effectively inhibits cell viability and promotes apoptosis in HCC cells. The mechanism of BETd-26-induced apoptosis is as follows: (1) mitochondrial membrane integrity is disrupted, which triggers apoptosis through intrinsic signaling; (2) cleavage of PARP and activation of caspase3 inhibit the transcription of the anti-apoptotic gene Mcl-1, which in turn increases the
expression of the pro-apoptotic Bcl-2 protein Bad; (3) Bad proteins bind to and antagonize anti-apoptotic Bcl-xL, Bcl-w, and Bcl-2 proteins. The study showed that Bad plays an essential role in BETd-260-triggered apoptosis.

The X protein (pX) of hepatitis B virus (HBV) acts as a multifunctional regulatory protein that cis activates the expression of many host genes. pX exists in nature as a dimer, which is involved in DNA repair and apoptosis. The C-terminal region of pX can maintain the stability of pX, called the unstable structural domain. The intermediate region of the protein located at amino acids (aa) 52–102 and the unstable region at aa103-154 form a fusion protein with GFP that can mediate proteasomal degradation of pX. Therefore, the unstable structural domain of pX can be considered as a novel E3 ligase recognition signal (degron). The N-terminal structural domain that regulates pX dimerization, known as the oligomerization domain, has no significant effect on the ubiquitination and stability of pX. pX has been reported to be essential for viral propagation and to promote progression to HCC in patients with chronic HBV infection. Moreover, pX proteins are prone to mutation and selective splicing in HCC. Therefore, Krissansen et al. designed and prepared a novel PROTAC that targets the degradation of pX and antagonizes its action. This PROTAC consists of a fusion of an oligomeric structural domain at the N terminus and an unstable structural domain at the C terminus. Moreover, they attached polyarginine cell-penetrating peptide (CPP) to the N terminus to enable PROTAC to achieve stronger cell permeability. Their study showed that PROTAC can efficiently degrade pX with either full-length or truncated unstable structural domains. Similar results were observed using the ODD domain of HIF-1α instead of the unstable structural domain. In addition, owing to the inclusion of CPP Xentry (Xentry, a new class of cell-penetrating peptide) at the N terminus, the oligomerization domain is inherently cell permeable and antagonizes pX-induced apoptosis. Therefore, PROTAC might have a dual destructive mechanism in pX. In conclusion, PROTAC, which antagonizes and disrupts pX, could be a novel strategy for the treatment of HBV or prevention of HCC.

**Application of PROTACs in cholangiocarcinoma**

Lu et al. reported the same antitumor effect of the BRD4 degrader ARV-825 in cholangiocarcinoma (CCA). BRD4 expression was upregulated in CCA tissues. ARV-825 effectively degraded BRD4 protein and was more efficient than the BRD4 inhibitors OTX-015 and JQ1. ARV-825 induced apoptosis and inhibited cell proliferation in CCA after rapid and sustained degradation of BRD4. c-Myc is a key downstream target of BRD4. c-Myc, an oncoprotein, is involved in many processes of cancer development and progression, including cell proliferation, senescence, apoptosis, and cell cycle progression.

**Application of PROTACs in pancreatic cancer**

Glucose-regulated protein, 78 kDa (GRP78) is a key chaperone factor of the ER. Under the high pressure of cancer cell proliferation with an increased demand for protein synthesis and folding, GRP78 is shed from the ER transmembrane receptor and exerts its pro-cancer effects through the following mechanisms. First, GRP78 activates protein kinase RNA-like ER kinase (PERK). Active PERK upregulates...
apoptotic genes through the eukaryotic initiation factor 2 (eIF2a)/transcription factor 4 (ATF4)/C/EBP homologous protein (CHOP)/growth arrest and DNA damage-inducible 34 (GADD34) signaling pathway, which ultimately leads to programmed cell death. Second, it can activate inositol-requiring enzyme-1 (IRE1) to promote the production of the splice variant X box-binding protein 1 (XBP1) and its translocation to the nucleus, which promotes ER chaperone gene transcription. This is a protective mechanism. Third, it activates transcription factor 6 (ATF6) to be shed from the ER transmembrane receptor. The shed ATF6 is transferred to the Golgi apparatus and acquires activity. Subsequently, active ATF6 is transferred to the nucleus to promote the unfolded protein response (UPR) transcription of GRP78, GRP94, protein disulfide isomerase (PDI), and XBP1. Finally, cancer cells use these UPRs to meet their survival and growth requirements. Therefore, Neamati’s group designed and prepared novel hydroxyquinolines targeting GRP78. YUM70, an analogue of hydroxyquinolines, inhibited pancreatic cancer cell growth in vitro. In addition, YUM70 promoted an increase in the level of ATF4 and phosphorylation of eIF2a, which in turn increased the expression of FAM129A (an ATF4 target gene with pro-apoptotic effects). FAM129A positively feeds back to regulate the PERK-eIF2a axis in pancreatic cancer and ultimately induces apoptosis. In addition, they synthesized YUM70-PROTAC with YUM70 as the warhead, named DX2-145. This is the first PROTAC that uses CRBN E3 ligase to degrade GRP78 in a dose-dependent manner.

Drug resistance in cancer is regulated by various factors such as EGFR overexpression and DNA repair enzymes. PARP regulates the repair of DNA single-strand breaks generated directly or during base excision repair. Some studies have reported that EGFR and PARP were upregulated in pancreatic cancer and non-small cell lung cancer (NSCLC), which demonstrated drug resistance. Inspired by the successful application of bispecific antibodies, Li et al. designed a dual PROTAC molecule based on CRBN/VHL, which can degrade two different targets simultaneously. They prepared a series of dual PROTACs, called DP-C 1–4 and DP-V 1–4. These dual PROTACs consist of two independent inhibitors, the EGFR inhibitor gefitinib and the PARP inhibitor olaparib, connected to the E3 ligands (CRBN/VHL) via a star-shaped linker that contains trifunctional natural amino acids. In the pancreatic cancer cell line SW1990, DP-C 1–4 can simultaneously target the ubiquitinated degradation of EGFR and PARP in a time-dependent manner. DP-V 1–4 also showed targeted degradation of EGFR and PARP in NSCLC cells. This is the first PROTAC targeting dual POIs and greatly expands the research field of PROTAC.

Cycle-related and expression-elevated protein in tumors (CREPT) is upregulated in pancreatic cancer and is strongly associated with low overall survival. CREPT overexpression promotes proliferation, colony formation, and migration of pancreatic cancer cells. CREPT exerts its pro-cancer effects probably through the following pathways: first, CREPT interacts with RNA polymerase II to mediate chromatin loop formation and activate the Wnt signaling pathway, subsequently inducing cyclin D1 transcription. Second, CREPT is closely associated with DNA damage repair. Third, CREPT promotes cell proliferation in the G1 phase. Fourth, CREPT is phosphorylated by Aurora B to regulate the expression of cell cycle protein B1, which ultimately promotes cell cycle progression from the G2 to M phase. Another study found that CREPT consists of an N-terminal regulation of nuclear pre-mRNA (RPR) domain and a C-terminal coiled-coil terminus (CCT) domain connected by a short hinge region. Therefore, Chang et al. chose the leucine-zipper-like motif (lysine 266 to valine 286) of the CCT structural domain as the CREPT ligand and linked it to the VHL ligand IYP(OH)AL via 6-aminohexanoic acid (AHX) to generate PROTAC-PRTC. In addition, the C terminus of PROTAC is connected to the transmembrane transport peptide RRK, which has strong cellular permeability. In pancreatic cancer cells, PRTC can combine with endogenous CREPT and induce the ubiquitous degradation of CREPT in a dose- and time-dependent manner. Ultimately, PRTC inhibited cell proliferation, colony formation, migration, and tumor growth in pancreatic cancer.

MYC is one of the important factors driving the development and progression of pancreatic cancer, and the transcriptional coactivator BRD4 can regulate the expression of key oncogenes MYC, KRAS, and Cavolin-2. Therefore, targeting BRD4 for degradation may be a new strategy for the treatment of pancreatic cancer. We have previously described that ARV-825 can efficiently degrade BRD4 in leukemia, gastric cancer, and cholangiocarcinoma. Therefore, to explore the role of ARV-825 in pancreatic cancer, ARV-containing polymeric nanoparticle (ARV-NP) was prepared by Saraswat et al. using a biodegradable PEG-poly lactic acid-co-glycolic acid (PLGA-PEG) matrix. ARV-NP is characterized by an enhanced permeability and retention (EPR) effect, long half-life, physical stability, controlled in vitro drug release, and negligible in vitro hemolysis. In vitro experiments demonstrated that ARV-NP can degrade BRD4 in pancreatic cancer cells. In turn, the expression levels of MYC and anti-apoptotic protein B cell lymphoma 2 (BCL2) were downregulated, and the expression level of the pro-apoptotic marker cleaved caspase-3 was upregulated. Eventually, cell proliferation, clone formation, and migration of pancreatic cancer cells were inhibited, and cell apoptosis was promoted.

KRAS-activating mutations are one of the drivers of most pancreatic cancers. Among them, the KRAS-RAF-MEK1/2-ERK1/2 pathway can induce transcription (e.g., induction of cell cycle protein D transcription) to promote cell cycle progression, ultimately leading to cancer development and progression. BRAF inhibitors and MEK1/2 inhibitors showed some efficacy in tumor suppression but had little effect on KRAS-mutated tumors. Therefore, Arribas’ group sought to explore the molecular mechanisms underlying pancreatic cancers that resist MEK1/2 inhibition and harbor KRAS mutations. They prepared pancreatic cancer cells resistant to MEK1/2 inhibitors that were also resistant to KRAS and ERK1/2 inhibitors. Their study showed that SLUG, a member of the SNAIL family of transcriptional repressors, is a key factor in pancreatic cancer cells acquiring
resistance to MEK1/2 inhibition. SNAI2, a gene encoding SLUG, is highly expressed in pancreatic cancer. SLUG separates tumorigenesis and development from the KRAS-RAF-MEK1/2-ERK1/2 signaling pathway, and also uncouples the regulation of D-type cell cycle protein D-cyclin 1 from MEK1/2. In conclusion, SLUG confers resistance and mesenchymal characteristics to MEK1/2 inhibition in pancreatic cancer and promotes the proliferation, invasion, and migration of pancreatic cancer cells. Furthermore, MEK1/2 inhibition induced the activation of the MEK5-ERK5 pathway, which also contributed to the upregulation of SLUG expression. Therefore, they concluded that targeting SLUG is an important strategy for the treatment of pancreatic cancer. They targeted SLUG degradation by using two independent strategies. One strategy is to inhibit the MEK5-ERK5 pathway, thereby inhibiting SLUG expression. The other is the design and preparation of PROTAC using a degradation tag (dTAG) system. SLUG–FKBP12F36V was generated by fusing the FKBP12 mutant protein (FKBP12P36V) to the C-terminal-tagged SLUG protein via the dTAG system. While SLUG–FKBP12P36V and CRBN were linked using dTAG-13, a compound that binds FKBP12P36V and CRBN, to induce the degradation of SLUG. The results of the study confirmed the efficacy of targeted degradation of SLUG in pancreatic cancers that are resistant to the KRAS-RAF-MEK1/2-ERK1/2 pathway.

Napabucasin has been extensively studied in cancer. The mechanism is described as follows: first, napabucasin has been reported to induce activation of the constitutive WNT/β-catenin signaling pathway. Second, napabucasin is a substrate for NAD(P)H quinone oxidoreductase 1 (NQO1), which is involved in ROS production and DNA alkylation, ultimately leading to apoptosis. Third, napabucasin inhibits protein synthesis by regulating eukaryotic initiation factor 4E (eIF4E). Fourth, napabucasin is considered to be an inhibitor of the signal transducer and transcription factor 3 (STAT3) signaling pathway. The use of napabucasin in esophageal, gastric, colon, and pancreatic cancers has shown some efficacy. It has been reported that napabucasin blocks STAT3 dimerization by binding to the SH2 structural domain of STAT3, thereby inhibiting the expression of STAT3. To explore the mechanism of STAT3 inhibition by napabucasin in depth, Maha et al. designed and prepared a series of PROTACs in pancreatic cancer cells. These consist of CRBN ligands and napabucasin linked by alkyl or PEG linkers. Among these, the pol amalgamated near XD2-149 was selected as the optimal PROTAC capable of inhibiting colony formation in pancreatic cancer cells. Its antitumor effect was superior to that of napabucasin. XD2-149 inhibited the interleukin (IL)6-dependent STAT3 signaling pathway and NQ1 protein synthesis in a proteasome-independent manner. In addition, XD2-149 was able to ubiquitinate proteasomal degradation of ZFP91. They found that part of the cytotoxicity of XD2-149 was dependent on the E3 ubiquitin protein ligase, ZFP91. ZFP91, which is involved in the FOXA1, HIF-1α, and nuclear factor κB (NF-κB) signaling pathways as an oncogenic protein, can also be ubiquitinated and degraded by XD2-149. XD2-149 regulates many biological processes through degradation of STAT3, NQO1, and ZFP91, including DNA replication, mismatch repair, signaling, telomere maintenance, hypoxia, UPR, inflammatory response, E2F target regulation, and apoptosis. In summary, napabucasin-based PROTAC has great potential for the treatment of gastrointestinal tumors.

Cell cycle protein-dependent kinase is a member of the serine/threonine kinase family. Cyclin-dependent kinase (CDK) 4/6 is activated upon binding to cyclin D, leading to positive feedback activation of the cell cycle RB protein family–E2F transcription factor–cell cycle gene CDK2-cell cycle protein E. Eventually, cell cycle progression is facilitated by a shift from the G1 to the S phase. Thus, targeting CDK4/6 can block cell cycle progression. Recently, a study designed and prepared a PROTAC that selectively degrades CDK6 in pancreatic cancer cells based on the different distribution of lysine residues exposed on the surface of CDK4 and CDK6. It consisted of palbociclib and CRBN E3 ligands connected by a linker. Palbociclib acts as an ATP-competitive kinase inhibitor that targets CDK4/6. The PROTAC selectively degraded CDK6 in human pancreatic normal ductal epithelial (HPNE) and MiaPaCa2 cell lines in a time-dependent manner, thereby blocking pancreatic cancer cell cycle progression.

It has been shown that B cell lymphoma extra-large (BCL-xL, an anti-apoptotic protein belonging to the BCL-2 family) plays a key role in the development and progression of pancreatic cancer and mediates gemcitabine resistance. Zhou’s group developed a novel PROTAC with superior BCL-xL degrading activity to the BCL-xL inhibitor ABT263 in leukemia, which was named DT2216. It consists of ABT263 and VHL ligands linked by a linker. They also found that targeting BCL2L1 (the gene encoding BCL-xL) enhanced the sensitivity of pancreatic cancer cells to gemcitabine, 5-flourouracil (5FU), and niraparib. Next, they determined that pancreatic cancer cells were highly sensitive to S63845, a selective inhibitor of MCL-1, and DT2216, a BCL-xL-specific PROTAC degradation agent. However, inhibition of MCL-1 leads to serious toxicities such as cardiotoxicity and hepatotoxicity. Moreover, dual inhibition of MCL-1 and BCL-xL can exacerbate these toxic side effects. Therefore, to enhance the efficacy of gemcitabine, a combination of gemcitabine and DT2216 was considered for the treatment of pancreatic cancer. Their study showed that the combination of gemcitabine and DT2216 induced apoptosis and inhibited tumor growth in pancreatic cancer cells, demonstrating synergistic antitumor effects. In addition, the degradation of BCL-xL by DT2216 increased the sensitivity of pancreatic cancer cells to gemcitabine and the dependence on MCL-1. Additionally, the levels of the apoptosis markers caspase 3 and PARP were also significantly increased. This suggests that the combination treatment of gemcitabine and DT2216 induced apoptosis in pancreatic cancer cells. Most importantly, in vivo experiment showed that the combination did not cause cardiotoxicity or hepatotoxicity in mice. In summary, the combination of gemcitabine and DT2216 in pancreatic cancer is a promising novel treatment.
Zhou et al. used a combination of sotorasib and DT2216 in NSCLC, CRC, and prostate cancer cell lines. Their study showed that combination treatment with sotorasib and DT2216 inhibited cell colony formation and induced caspase-mediated apoptosis. DT2216 enhances the sensitivity of cancer cells to sotorasib. Furthermore, they found that the mechanism of sotorasib-induced apoptosis in cancer cells may be achieved by maintaining the stability of BH3-only pro-apoptotic proteins (BIM, BMF, and PUMA). Overall, DT2216 demonstrated strong antitumor synergistic effects in combination with other drugs in oncology. DT2216 is currently in a phase I clinical trial in patients with relapsed/refractory malignancies (NCT04886622).

**Application of PROTACs in colorectal cancer**

The Wnt/β-catenin signaling pathway is aberrantly activated in many types of cancers. The activated Wnt/β-catenin signaling pathway allows β-catenin to be deposited in the cytoplasm and translocate to the nucleus, ultimately promoting cell proliferation and differentiation. Such phenomena have also been observed in colorectal cancer. Therefore, targeting the Wnt/β-catenin pathway is considered an important route for cancer therapy. Chen’s group and Verdine’s lab designed two stapled peptides, SAHPA1 and xStAx, based on the same Axin-derived peptide motif that can penetrate cell membranes and bind to β-catenin. Among these, SAHPA1 activates the Wnt/β-catenin signaling pathway. In contrast, xStAx inhibits the Wnt/β-catenin signaling pathway by blocking β-catenin-TCF interaction in the nucleus. Based on this study, Chen’s group synthesized PROTACs that target the degradation of β-catenin, called xStAx-VHLL. xStAx-VHLL consists of xStAx (β-catenin ligand), a VHL ligand coupled to an Ahx chemical junction. This study demonstrated the ability of xStAx-VHLL to sustainably degrade endogenous β-catenin in cancer cells and intestinal organoids in a dose-dependent manner. In addition, xStAx-VHLL inhibits Wnt/β-catenin and suppresses Wnt downstream signaling targets, including Lgr5, Axin2, cyclin D1, and MYC. Ultimately, xStAx-VHLL exhibited inhibitory effects on colorectal cancer cell proliferation, tumor growth, and intestinal organoid survival in the intact Wnt/β-catenin signaling, APC deficiency, and β-catenin mutant systems.

PROTAC A1874 is an idasanutin-based degrader of BRD4. A1874 can degrade BRD4 and upregulate the expression of the tumor suppressor p53. It consists of the BET inhibitor JQ1 and the MDM2 inhibitor idasanutin, linked by PEG. In the colon cancer cell line HCT116, A1874 effectively degraded BRD4 and downregulated the transcription factor c-Myc, which drives cell proliferation. In addition, A1874 enhanced the stability of the tumor suppressor p53 and increased expression level of its effector protein p21 (CIP1/WAF1). P53, a MDM2 target, can cause cell cycle arrest in cells with damaged DNA and/or induce the activation of apoptotic signals. This suggests that A1874 is capable of exerting its effects via a BRD4-dependent or BRD4-independent pathway. Another study showed that the production of ROS in the non-dependent pathway of A1874 plays an equally important role. The antioxidant N-acetylcysteine (NCA) was used to attenuate A1874-mediated colon cancer cell death or apoptosis. Their study also confirmed that A1874 induces the ubiquitinated degradation of BRD4 and downregulates the expression of BRD-dependent genes (c-Myc, Bcl-2, and cyclin D1). The cytotoxicity of A1874 was closely linked to P53 stability and ROS production. A1874 significantly inhibited colon cancer cell proliferation, viability, cell cycle progression, migration, invasion, and tumor growth through the above pathways. Therefore, nutlin-based PROTAC may provide a new strategy for future cancer therapy owing to its dual oncogenic mechanism.

Another study on the induction of BRD4 degradation showed that the BRD4 inhibitor JQ1 downregulated MYC mRNA levels and MYC protein expression in colorectal cancer cells by inhibiting BRD4 expression, ultimately suppressing CRC cell proliferation. Wiener et al. cross-sectionally compared the effects of JQ1, dBET1, and MZ1, which target BRD4, for downregulation of MYC in colon cancer. Among them, dBET1 and MZI are PROTACs composed of JQ1 and thalidomide/VHL ligand via a linker, respectively. Their study revealed that dBET1 and MZ1 efficiently degraded BRD4 in an E3 ligase-dependent manner, which in turn inhibited the transcription and expression of MYC mRNA. Furthermore, the authors investigated the mechanism of anti-dBET1 treatment in colon cancer cells by synthesizing anti-dBET1 LS174tβET1-R cells. Their study showed that both CRBN mRNA and protein levels decreased in dBET1-resistant LS174tβET1-R cells, suggesting that depletion of CRBN may induce the development of dBET1 resistance. However, JQ1 and MZI still affected LS174tβET1-R cells, exhibiting inhibition or degradation of BRD4 and a significant decrease in MYC mRNA and MYC protein expression.

Natarajan et al. designed and prepared aminopyrazole-based PROTAC, which selectively degrades CDK9 in colorectal cancer HCT116 cells. The PROTAC was synthesized using a linker connecting the CRBN ligand thalidomide and a selective CDK inhibitor containing aminopyrazole. CDK9 acts as a member of the CDK family of serine-threonine kinases. It is involved in the cell cycle and gene transcription by interacting with cell cycle proteins. CDK9 degradation by PROTAC inhibits the phosphorylation of Ser2 on RPB1, thereby suppressing the expression of the pro-survival protein Mcl-1. Then, they further refined the PROTAC. They found that changing the linker length and composition could have a dramatic effect on CDK9 degradation. Modified PROTAC downregulates Mcl-1 expression and increases the sensitivity of pancreatic cancer MiaPaCa2 cells to the Bcl2 inhibitor Venetoclax, following time- and dose-dependent degradation of CDK9. Simultaneous inactivation of Mcl-1 and Bcl-xL induces apoptosis. Thus, aminopyrazole-based PROTAC offers a novel approach for the treatment of pancreatic and colorectal cancer.

The histone deacetylase (HDAC) family of enzymes plays a key role in epigenetic regulation. Thus far, 18 HDACs have been documented. Of these, the sum of HDAC 1, 2, and 3 account for approximately half of the cellular deacetylases, which are localized in the nucleus. In addition, HDACs play a catalytic role in...
DNA damage can induce programmed cell death and tumor cell clearance. However, it has been shown that DNA repair can survive chemotherapy-induced DNA damage in tumor cells through many endogenous mechanisms, including mismatch repair, base excision repair, and nucleotide excision repair (NER). Excision repair cross-complementation group 2 (ERCC2) is a member of the NER proteins family that is essential for transcription initiation, DNA damage recognition, and NER. One study found that ERCC2 expression was significantly downregulated in colon cancer cells after treatment with the histone deacetylase 3 (HDAC3) inhibitor turnip sulfur (SFN) in combination with the BET inhibitor JQ1. Therefore, Sabeeta et al. used ERCC2 as a therapeutic target for colon cancer to investigate a more effective combination therapy strategy. Their study showed that overexpression of ERCC2 resulted in a low survival rate in colon cancer cells after treatment with the histone deacetylase 3 (HDAC3) inhibitor turnip sulfur (SFN) in combination with the BET inhibitor JQ1. Therefore, Sabeeta et al. used ERCC2 as a therapeutic target for colon cancer to investigate a more effective combination therapy strategy. In summary, PROTAC-mediated degradation of histone deacetylases in the co-degrading complex may provide a new strategy for targeted epigenetic therapy of colon cancer.

Programmed cell death ligand 1 (PD-L1) is essential for tumor immune evasion. Tumor cells evade T cell immunity through the PD-L1/programmed death protein 1 (PD-1) signaling pathway.

Immune checkpoint blocking antibodies against PD-1, PD-L1, and cytotoxic T lymphocyte-associated protein 4 (CTLA4) have been widely used in clinical oncology therapeutics. However, anti-PD-L1 has no significant efficacy in several solid tumors. Therefore, an in-depth exploration of the regulatory mechanism of PD-L1 to discover other, more effective therapies to inhibit PD-L1 function would be beneficial for cancer patients. BMS-37 (a small diaryl ether molecule) can bind to the extracellular structural domain of PD-L1. Wang et al. found that the flexible ethanediamine side chain of BMS-37 could bind to proteins. Therefore, they used the flexible ethanediamine chain of BMS-37 and CRBN ligand to generate a PROTAC that could target PD-L1 protein degradation via a linker connection. Among them, PROTAC molecule 21a is less toxic and induces proteasomal degradation of PD-L1 in the cytoplasm of a variety of cancer cell lines, including colon cancer cell lines MC-38 and SW-480, in a dose- and time-dependent manner. A decrease in PD-L1 protein levels was accompanied by a decrease in PD-L1 mRNA levels. It was shown that PROTAC molecule 21a could down-regulate PD-L1 protein levels in cancer cells and induce chemotaxis of CD8+ T cells in MC-38 cells, ultimately inhibiting tumor growth in vivo. In summary, PROTAC targeting PD-L1 might play an anti-tumor role in solid tumors with poor anti-PD-L1 efficacy. However, this also requires further validation by future studies.

During eukaryotic gene expression, spliceosomes are required to shear introns to convert pre-mRNA to mature mRNA. The spliceosome consists of a multiprotein complex of five small nuclear RNAs (snRNAs) and associated proteins (e.g., splicing factor 3B, SF3B). SF3B subunit 1 (SF3B1) is the largest subunit of SF3B and one of the core constituents of the spliceosome. SF3B1 mutations have been reported to be involved in the development and progression of multiple cancers. SMIs targeting SF3B1 have been found to block splicing and induce apoptosis.
maintain human induced pluripotent stem cells (hiPSCs). Then, they identified SF3B1 as one of the key downstream targets of O4I2. Therefore, they developed PROTAC-O4I2 to target SF3B1. PROTAC-O4I2 consists of thalidomide and O4I2 connected via a linker. PROTAC-O4I2 induced ubiquitinated proteasomal degradation of SF3B1 in a dose-dependent manner in HEK293, HEPG2, K562, and HeLa cell. Furthermore, they showed that PROTAC-O4I2 exerted antitumor activity by interfering with the proliferation and maintenance of stem cells in a Drosophila intestinal tumor model. As a result, the growth of Drosophila intestinal tumors was inhibited.

CONCLUSIONS AND PERSPECTIVE

Since its discovery in 2001, PROTAC has become a useful technique for targeted degradation of proteins and can be used as an alternative approach in cancer treatment. PROTAC exhibits many other advantages in addition to its unique chemical degradability: (1) PROTAC overcomes resistance to SMIs or mAbs caused by mutations, overexpression, and adaptation of target proteins. (2) PROTAC targets the degradation of non-drug targets or drug-resistant targets, including nuclear receptors, transcription factors, and folded proteins. (3) PROTAC has good oral bioavailability, and ARV-110 and ARV-471 were administered orally in clinical trials. (4) PROTAC is highly targeted and tissue specific. Various human tissues express different E3 ligases, which may reduce the side effects of PROTAC and improve its safety. (5) PROTACs have good controllability. They can modulate their activity by phosphorylation activation or photoswitching to reduce their off-target effects. Despite these advantages, PROTACs have some limitations. Their efficacy depends not only on the POI and E3 ligase ligands but also on the chemical properties and the length of the linkers. The POI, E3 ligase ligands, and linkers determine the size of the PROTAC molecule, affinity for forming ternary complexes, cell permeability, compound stability, and spatial orientation. This makes the process of designing and preparing an effective PROTAC tedious. In addition, POI is not only found in diseased tissues but may also be present in normal tissues. This can lead to off-target effects and serious adverse reactions. Furthermore, mutation or depletion of E3 ligase may lead to resistance to PROTAC. Therefore, a proper structural design is key to effective PROTAC development.

The developmental potential of the PROTAC technique has not yet been fully explored. As a new drug modality, PROTAC eliminates a resistance to PROTAC. Therefore, a proper structural design is key to effective PROTAC development.

An increasing number of PROTACs have been successfully developed in gastrointestinal cancers. They can block multiple oncogenic signaling pathways to inhibit tumorigenesis and progression. However, third-generation PROTACs controlled in a spatiotemporal manner have not been widely used for gastrointestinal cancers. In addition, many PROTACs have been used in clinical trials for breast, prostate, lung, and hematologic cancers. Unfortunately, there are no reports of PROTAC in clinical trials for gastrointestinal cancers. Therefore, the PROTAC technique needs to be further investigated to develop more effective heterobifunctional degraders of PROTACs and to provide new, safe, and effective targeted drugs for patients with gastrointestinal cancers. We believe that controlled PROTACs will become a reality in the future and provide new antitumor drug options for patients with cancer.

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AUTHOR CONTRIBUTIONS

Y.C., Q.Y., J.X., and Y.Z. were responsible for the review of the literature. Y.C., Q.Y., and J.X. wrote the manuscript. F.D., Y.Z., M.L., and J.S. edited the manuscript. W.L., X.L., M.C., and Z.W. drew the tables and pictures. L.T., X.W., R.D., and H.C. edited the tables and pictures. H.C., Y.D., Q.W., and Z.X. designed the study and made valuable discussions and revisions to the manuscript. All authors contributed to the article and approved the submitted version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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