Proteolysis Targeting Chimeras (PROTACs): Emerging Therapeutics for Pancreatic Cancer

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Abstract: Proteolysis targeting chimeras (PROTACs) is a newly developed protein-knockout strategy, which degrades target protein by ubiquitination and ligation. It has shown a promising effect in both cancer and immunotherapy. Pancreatic cancer is one of the most progressive cancers. Current therapies for it include surgery and radiation therapy, but both have low survival rates, as illustrated in this paper. Compared to traditional therapies for pancreatic cancer, PROTAC has the benefit of minor side effects and negligible drug resistance. In recent years, breakthroughs in pancreatic cancer are accomplished through PROTAC. In this paper, the mechanism of PROTAC is summarized in full detail, current targets of PROTAC are analyzed and the potential directions of future research are given. Targets specifically for pancreatic cancers, such as Napabucasin, a STAT3 inhibitor, with napabucasin-based PROTAC, the mechanism of napabucasin are under exploration and their progress is shared in this article. Moreover, BDR4 and CREPT, which are common targets in many different cancers, are also included for their outstanding performance in PROTACs' drug delivery.

1. Introduction

Proteolysis targeting chimeras PROTACs is a promising molecular technology targeting the proteasome level. PROTACs aim to degrade pathologic proteins through ligation and ubiquitination. This molecular technology has shown significant progress in some gene products, including BCR-ABL [1], which is responsible for many different types of leukemia. The applications of PROTACs in different cancers are being studied including pancreatic cancer, which usually refers to pancreatic adenocarcinoma. It usually has no symptoms at the early stage of onset and will progress to jaundice, back pain, and unexplainable weight loss. Multifactorial approaches are associated with pancreatic cancer onset, both genetic and environmental factors are determined to have associations with pancreatic cancer [2].

Alcohol and smoking are believed to increase pancreatic risks. The traditional treatment for pancreatic cancers is surgery, which is limited to the stages and tumor positions. If the tumor on the mesenteric artery or abdominal aorta exceeds 180 degrees or metastasis is involved, the tumor is inoperable. Adjuvant treatment including chemotherapy using FOLFOROMPX plan, gemcitabine, and taxol has shown limitations of drug resistance and contradictions. Due to physical differences, current guidelines do not recommend applying adjuvant treatment to all patients. Conventional treatments are limited and ineffective in many patients due to their chance of operation, the effectiveness of chemo-radiotherapy, and rapid metastasis due to sufficient blood circulation. The potential effectiveness of PROTACs is being analyzed and showing great potential benefits to the patients. Some targets have been proposed, such as CDK4, TP53, and BRD4. As table 1 showed, many germline mutations of pancreatic cancer also contribute to the difficulty of treatment. This paper will

review the outcomes of PROTACs utilization upon patients who have pancreatic cancer; challenges and insight of future PROTACs development will be analyzed as well.

Table.1. Germline mutations relate to pancreatic cancer risk [17]

Disease	Gene	Pancreatic Cancer Risk (%)	Risk to Normal Population (folds)
Peutz-Jeghers Disorder	STK11	11-36 (at the age of 65-70)	132
Familial Pancreatitis	PRSS1, SPINK1, CFTR	40-53 (at the age of 70-75)	26-87
Melanoma-Pancreatic Cancer Syndrome	CDKN2A	14-17 (at the age of 70)	20-47
Lynch Syndrome	MLH1, MSH2 (MSH6)	4 (at the age of 70)	9-11
Mammary carcinoma/oophoroma Syndrome	BRCA1, BRCA2	1.4-1.5 (Female, at the age of 70) 2.1-4.1 (Male, at age of 70)	2.4-6.0

2. PROTACs and other therapies for pancreatic cancer

2.1 Current therapies

Pancreatic cancer is one of the most lethal solid tumors. It is now the third leading cause of cancer-related mortality, and has been predicted to become the second leading cause in the next decades. These years, pancreatic cancer has showed a drastic increase in the world-wild trend, either of incidence or mortality. The low efficiency in prevention and treatment and low diagnosis in the early stage lead to few treatment options, and eventually reduces the patient's survival (9% surviving patients in five years, only 20% patients surviving within one year) [3].

Surgery for pancreatic cancer includes removing all or part of the pancreas, depending on the severity of the tumor. The area of healthy tissue around the tumor, which is called margin, is also removed. The goal of surgery is to have “clear margins” with no cancer cells in the edges of the healthy tissue removed.

Only about 20% of people diagnosed with pancreatic cancer can have surgery because most pancreatic cancers are found after the cancer cells have already spread. Surgery for pancreatic cancer may be combined with systemic therapy and/or radiation therapy. These therapies may sometimes be used before surgery to shrink a tumor. This is called neoadjuvant therapy or pre-operative therapy. After neoadjuvant therapy, the tumor is re-staged before planning a surgery, which is reaccessing the size and effect of the tumor.

Sometimes, the surgeon may choose to start with a laparoscopy. During a laparoscopy, several small holes are made in the abdomen and a tiny camera is passed into the body while a patient receives anesthesia. During this surgery, the surgeon can find out whether cancer has spread to other parts of the abdomen. If it has, using surgery to remove the primary tumor is generally not recommended. A substitute way is called the Whipple procedure. This surgery may be done if the cancer is located only in the head of the pancreas. This is an extensive surgery in which the surgeon removes the head of the pancreas and the part of the small intestine called the duodenum, as well as the bile duct and stomach. Then, the surgeon reconnects the digestive tract and biliary system. Another surgery is called Distal pancreatectomy, which is commonly done if the cancer is located in the tail of the pancreas. In this surgery, the surgeon removes the tail and body of the pancreas, as well as the spleen. If cancer has spread throughout the pancreas or is located in many areas in the pancreas, a total pancreatectomy may be operated. A total pancreatectomy is the removal of the entire pancreas, part of the small intestine, a portion of the stomach, the common bile duct, the gallbladder, and the spleen. Side effects of surgery

include weakness, tiredness, and pain for the first few days after the procedure. Other side effects caused by the removal of the pancreas include difficulty digesting food and diabetes from the loss of insulin produced by the pancreas.

Radical surgery is the best treatment for patients with pancreatic cancer, however, a very low percentage of the patients can have anatomically resectable treatment, partly because of the late diagnosis. Besides, the low survival rate and high possibilities of recurrence in post-operative patients remain a challenge in oncology [4]. Radiation therapy uses high-energy x-rays to destroy tumor cells. The most common type of radiation treatment is called external-beam radiation therapy by giving radiation from a machine outside the body.

Side effects from radiation therapy may include fatigue, mild skin reactions, nausea, and loose bowel movements. Most side effects speed away soon after treatment is completed.

In recent years, next-generation genome sequencing (NGS) opens a new chapter in the treatment of pancreatic cancer. Identifying mutated genes and thus inventing targeted molecules to treat them is a hot trend (table 2) [5].

Table. 2. Potential targets and treatments in pancreatic ductal adenocarcinoma

Targets	Diseases or Syndrome	Treatment/ intervention	Phase	Reported frequency
KRAS [5]	Metastatic PDAC	Mesenchymal stromal cell-derived exosomes with KRAS siRNA	1	80%-90%
TP53 [5, 15]	Li-Fraumeni Syndrome	APR-246 (Cysteine binding compound) COTI-2	1	80%
BRCA1/2 [5, 17]	Hereditary breast and/or ovarian cancer	crosslinking agents PARPi	1, 2, 3	5-10
CDK4 [5]	FAMMM syndrome	Palbociclib, PD-0332991	1	unknown
CDKN2A [5, 17, 19]	Familial atypical multiple mole and melanoma syndrome	CDK4/6 inhibitor		80%
NTRK inhibitor	NTRK fusion-positive solid tumors	Entrectinbi, Larotrectinib	2	<1
ALK inhibitor	Advanced solid tumors	Ceritinib	1	unknown
BRCA, HRD	Hereditary breast-ovarian cancer syndrome	PARPI, PLATINUM	1, 2, 3	5
TRK/ROS1	unknown	Entrectinib, Larotrectinib	2	unknown
STAT3 and cancer cell stemness inhibitor	Metastatic PC	Paclitaxel and gemcitabine plus or without napabucasin	3	unknown
MMRd	unknown	Immunotherapy	1, 2	unknown

2.2 PROTACs for pancreatic cancer

2.2.1 Mechanism of PROTACs

PROTAC is a prominent immunotherapeutic technology for the degradation of protein of interest. PROTAC is a bifunctional molecule consisting of a ligand of the protein of interest (POI) that is made of a small-molecule inhibitor and an E3 ubiquitin ligase that allows covalent binding. PROTACs recruit E3 ligase for proteasome-mediated degradations. PROTACs show significant POI reduction along with nucleic acid-based gene knockdown technologies and have been utilized in multiple diseases, including Chronic Myeloid Leukemia, BCL2, prostate cancers, pancreatic malignant tumors,

etc. There are around 50 PROTACs targeting proteins that have been successfully developed showing positive clinical outcomes [6].

2.2.2 Napabucasin-based PROTAC: XD2-149

Pancreatic cancer is one of the deadliest cancers, of which only 10% to 15% accounts for the resectable stage. Besides, pancreatic cancer is associated with high possibilities of metastasis and an extremely low rate of survival. Early symptoms of pancreatic cancer are usually nonspecific, and symptoms occur in late progression, so most patients are diagnosed at the disseminated stage when traditional surgical treatment cannot be available [7]. The traditional treatment for pancreatic cancer also involves radiotherapy and chemotherapy, for patients who can receive surgical resection, may also suffer from recurrence and chemotherapy resistance. Later, more emerging studies reveal mutation of certain molecules can induce PC, such as KRAS, TP53, and CDKN2A [7]. Then treatments aimed to inhibit these molecular abnormalities have been invented, which are also combined with chemotherapy, for example, Erlotinib and Larotrectinib [8]. However, the poor outcome showed the survival time for patients with pancreatic cancer almost does not change. Besides, radiotherapy and chemotherapy will also diminish patients' quality of life. And the detectable technology for these markers has low specificity and sensitivity.

STAT3 is a signal transducer and transcription factor, of which activation has already shown a strong relationship with the metastasis and proliferation in cancer cells. This characteristic makes STAT3 become one of the attractive targets in the oncology field. Napabucasin, also known as BBI608, was first discovered in 1982 from compounds obtained from plants, with anti-cancer activity, initially, it was called Naphthoquinone 2-acetyl benzo[f]benzofuran-4, 9-dione (NPQ). However, it did not arouse great attention until 2009, when Boston Biomedical, Inc renamed it Napabucasin [8].

After a series of clinical trials, researchers gradually realized Napabucasin can inhibit the STAT3 signaling pathway. Even though Napabucasin is known for inhibiting STAT3, the exact mechanism is still unclear. One attempt to explore this mechanism is SD-36, first reported in 2019 with STAT3 degrader in CRBN PROTACs [8].

In 2021, Hanafi groups reported that the designed Napabucasin-based PROTAC XD2-149 degrades the E3 ubiquitin-protein ligase. Hanafi groups tried various designed structures based on the optimization of linker length and compositions, the attachment positions of the linker to napabucasin, and kinds of E3 ligase ligands. They did experiments in both VHL PROTACs and CRBN-based PROTACs and found CRBN-based PROTACs were more effective. They mainly did explorations in BxPC-3 cells, which contain high-level protein of STAT3. They created XD2-149, which later they found was significantly inhibiting the STAT3 transcription pathway and reducing expression of STAT3 and related proteins. Besides, XD2-149 also shows its ability as a degrader of ZEP91, which is highly expressed in pancreatic cancer [8].

Comparing the traditional tumorectomy and early molecule inhibition, Hanafi groups, after synthesizing various PROTACs compounds, created XD2-149, which can inhibit the IL6 with STAT3 signaling pathway and be effective in proteasome-dependent degradation of ZEP91. This Napabucasin-based PROTAC is very promising in the degradation of STAT3. Even though ZEP91 plays a big role in the development of pancreatic cancer progression, so far, no related therapies targeting ZEP91 have emerged. Some researchers have pointed out CRBN-based PROTAC involving GSPT1 and ZEP91, however, no anti-tumor cancer therapies have been figured out [8].

2.2.3 Other targets of PROTACs: BRD4

BRD4, a member of the bromodomain family [9], has been identified as a driving factor of tumorigenesis. BRD4 has a transcriptional regulatory mechanism that can activate MYC gene transcription, which leads to abnormal cell proliferation. Potential PROTACs could target phosphorylation sites of MYC protein, and further cause ubiquitination and degradation. An effector ligand, pomalidomide has shown to be effective in degrading POI. Newly developed PROTACs ARV825 has two ligands: one is OTX015, also known as Thienodizepine targeting BRD4 protein, the other is phthalimide or pomalidomide that specifically target E3 ubiquitin ligase cereblon. The ligand system relocates BRD4 to CRBN and initiates prolonged degradation of BRD4 and subsequently inhibits the MYC gene expression to halt abnormal cell proliferation [10]. ARV has shown significant

outcomes targeting vemurafenib-resistant melanoma due to its cytotoxic, apoptotic, and antimetastatic activity [11]. Currently, a 2D and 3D cell array shows antitumor effects induced by ARV. A current issue that is being addressed is the delivery route to the tumor sites. Their metabolic pathways are different from conventional small molecules that are capable of inducing protein degradation. Apart from pharmacokinetics, metabolism pathways, permeability, the delivery route is a significant challenge. PROTACs have low oral bioavailability due to they are developed based on organic molecules, which are unlikely to be taken orally. It must be directly delivered to the pancreas, however, ARV-based PROTACs have low aqueous solubility, it turns out to be challenging to design its intravenous formulation. Once the oral bioavailability has been improved or administration pathways have been determined, ARV-mediated degradation could be a novel treatment targeting abnormal BRD4 proteins or MYC oncogene products on KRAS-mutant patients. One of the potential delivery solutions is developing nano careers of peptic and other organic anticancer drugs. Based on the current research, chitosan nanoparticles (CNP) have been employed as a drug delivery system for many peptic drugs and organic anticancer drugs [12]. It can escort drugs to penetrate through the GI mucous membrane (bypass the efflux pump of P-glycoprotein). However, the oral bioavailability still needs further investigations since the First Pass Effect of ChNP is unclear and ARV is metabolized by the liver CYP3A4 system.

2.2.4 Other targets of PROTACs: CREPT

CREPT is an RPRD (regulation of nuclear pre-mRNA-domain-containing) protein containing C-terminal domain (CTD)-interacting domain (CID), which mediates the binding to the CTD of Rpb1, the largest subunit of RNA polymerase II (RNAPII) [13]. It is highly expressed in pancreatic cancer and is associated with poor disease-free survival. Overexpression of CREPT promotes the proliferation of pancreatic cancer cells. Ma and his team designed cell-permeable peptide-based proteolysis targeting chimera (PROTAC) based on the homodimerized leucine-zipper-like motif in the C-terminus domain of CREPT to induce its degradation *in vivo* [14]. It turns out that the PROTAC with CREPT C-terminus domain inhibits cancer cell colony formation, cell proliferation, and motility in pancreatic cancer cells, which is comparable to that of deleting the CREPT oncogene. Therefore, the researchers suggest that their PROTAC can induce degradation of CREPT that leads to inhibition of tumor growth, which is promising for the development of new drugs against pancreatic cancer.

3. Conclusion

Current treatments for Pancreatic Cancer include surgery and radiation therapies. However, these treatments have significantly low survival rates often due to the metastasis of pancreatic cancer. Therefore, applying a systemic therapy that removes all potential tumor cells is promising. PROTAC is being widely studied for its low toxicity and high efficiency of targeting and digesting proteins responsible for tumor cell formation. Hanafi groups, after synthesizing various PROTACs compounds, created XD2-149, which can inhibit the IL6 with STAT3 signaling pathway and also be effective in proteasome-dependent degradation of ZEP91, have high cytotoxicity dependent on ZEP91 and is very promising in degradation of STAT3. In addition, ARV PROTACs system acting on BRD4 has proven to be a positive clinical approach for pancreatic cancers since it could inhibit the MYC protein signaling pathways without interfering with normal protein functions.

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