Progress of immunotherapy in the treatment of pancreatic cancer

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Abstract. As a highly aggressive malignancy characterized by a high rate of morbidity and mortality, pancreatic cancer (PC) is a solid tumor ranking seventh among all cancer deaths. Since clinical symptoms are hidden and effective early diagnostic methods are unavailable, it is common that patients with PC are at an advanced stage once diagnosed and cannot be radically treated by surgical resection. The universal existing anticancer treatments are chemotherapy and radiotherapy, but the therapeutic effect of PC is not obvious. Recent researches witness encouraging success in immunotherapy used for hematologic tumors which resulted in immunotherapy becoming the hotspot of cancer treatment over the past few years. Many researchers started to turn their attention to its application in other cancer treatments and therapy in melanoma and non-small cell lung cancer (NSCLC) has made great progress. Through further research on the mechanism of immunotherapy, many novel cancer treatments start to emerge, such as adoptive T cell therapy, immune checkpoint inhibitors, tumor vaccines and oncolytic viruses. Many clinical trials proved that combining immunotherapy with traditional therapy, like surgery, chemotherapy, and radiotherapy has a remarkable effect on the treatment of patients with PC. Individualized, combined, and precise therapy may be a promising direction for future immunotherapy in PC. The current understanding of the occurrence and development of PC, the progress in immunotherapy in cancer treatment, and the prospect of immunotherapy for PC will be briefly introduced in this review.

Keywords: Pancreatic cancer, Immunotherapy, CAR-T cell therapy, Immune checkpoint inhibitor, Tumor vaccine, Oncolytic virus.

1. Introduction

Although cancer treatment has made much progress, the five-year survival rate of pancreatic cancer (PC) is still alarming, only 10%. Due to its high mortality rate, it ranks seventh among all cancer deaths [1]. The easy invasion of surrounding blood vessels and hematogenous metastasis are important factors of its high degree of malignant. Since effective early diagnostic methods are unavailable, such as highly specific and sensitive tumor markers or iconography, more than 90% of patients with PC have the diagnosis at an advanced stage with metastasis and have a poor prognosis. Therefore, resectable surgery in combination with adjuvant therapy is the main treatment for PC. However, the patients who are suitable for surgery account for only 20% of all confirmed patients, and about 80% of these patients had a recurrence and died after surgery.

In addition to age and genetics, smoking, obesity, diabetes, chronic pancreatitis, and other factors are all related to the occurrence of PC [2]. Smokers were found susceptible to PC and the morbidity was associated with the amount of smoking for a lifetime, dropping to the level of a never smoker after 15 years since quitting smoking. The researchers hypothesize that carcinogens in tobacco enter the biliary tract and then flow back into the pancreatic duct, causing carcinogenic mutations at a specific site. In addition, the morbidity of PC is 2.89 times higher for diabetics than for the healthy group. The increase in insulin level in diabetics may be the pathogenesis of diabetes-induced PC. After the activation of insulin-like growth factor 1 (IGF-1) receptor, pancreatic stellate cells proliferate and differentiate abnormally, leading to tumor formation [3]. In 2008, Jones et al. conducted the first full exon sequencing of PC and revealed gene point mutations that alter 12 cellular pathways, among which KRAS, TP53, SMAD4, and CD-KN2A were the four main driving mutants [4]. Most mutations were found in primary and metastatic sites and it took about 20 years from the

first mutation to the onset of metastasis. As the mutations accumulate, a few mutations provide cell growth advantages and drive normal cells to transform into tumor cells, forming a malignant tumor through infiltration, invasion, and metastasis, as shown in Fig.1.

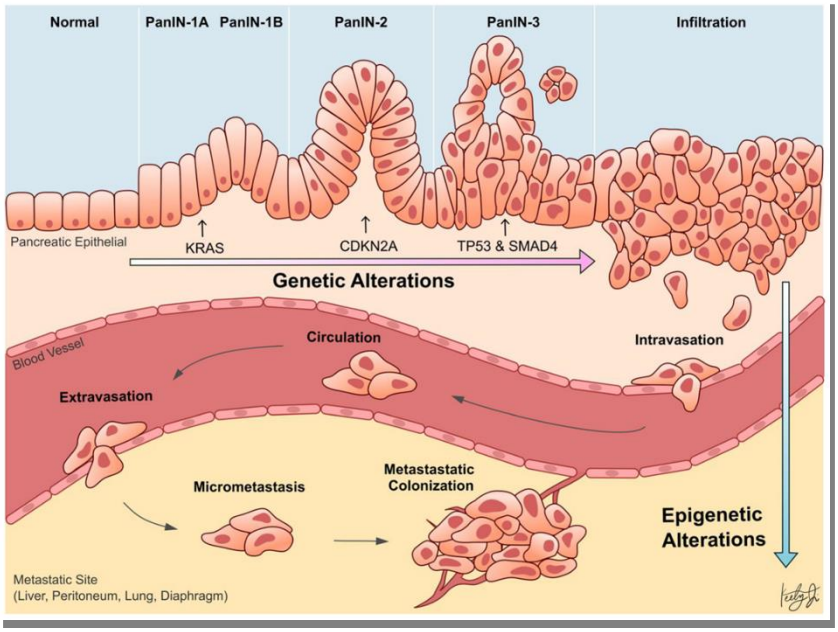


Figure 1. The progression and metastasis of PC.[5]

Cells that proliferate abnormally activate pancreatic stellate cells, change them into myofibroblast phenotype, and produce a considerable number of extracellular matrix proteins, causing the formation of a dense fibrotic matrix environment that blocks the infiltration of CD8+ tumor-infiltrating lymphocytes (TILs) [6]. The low invasion of immune cells in the tumor microenvironment (TME) of PC becomes one of the main factors to limit the curative effect of immunotherapy. Therefore, researchers have conducted extensive studies on immunotherapy of PC patients over recent years. In this article, the current understanding of the occurrence and development of PC, the progress in various immunotherapies, and the prospect of immunotherapy for PC will be reviewed.

2. Immunity and Tumor

2.1. Tumor immunosuppression

The human immune system serves as a defender to recognize and remove abnormally growing cells, such as tumor cells. One of mechanisms that tumor cells escape from the attack of immune system is to decrease the expression of tumor-associated antigens (TAAs) or temporarily lose them by endocytosis to reduce their immunogenicity and evade the attack of the immune system. Tumor cells can alter the interaction between major histocompatibility complex (MHC) and antigenic peptides to hinder the specific recognition and binding between T cell receptor (TCR) and MHC-antigenic peptide complexes. In addition, tumor cells down-regulate costimulatory molecules and form various coinhibitory molecules to block the immune response and even induce immune tolerance. The binding between the Fas receptor and its ligand FasL is reported to mediate apoptosis. Tumor cells can express fewer Fas to protect themselves from attack or actively express FasL to kill the infiltrating effector cells with Fas receptors. Also, it is reported that heat shock proteins induced by stressors such as high temperature are overexpressed in the majority of human cancers, involving the proliferation, invasion, and differentiation of tumor cells with significant anti-apoptosis effect, which can be regarded as promising tumor markers and therapeutic targets [7].

2.2. Tumor Immunosuppressive Microenvironment

Cytokines are involved in the process of immune cell recruitment as shown in Table 1. The TME with the formation of the abnormal blood vessel and acidic hypoxia recruits immunosuppressive cells like regulatory T cells (Tregs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and secretes immunosuppressive factors like vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and interleukin (IL) [8].

Table 1. Tumor microenvironment-related cytokines that recruited inflammatory/immune cells.[9]

Inflammatory/immune cells	Related cytokines that recruited cells
DCs	IFN-ß, IFN-γ, IL-12, IL-18
NKs	TNF-α, IFN-γ, IL-2, IL-12
TAMs	TNF-α, TGF-ß, IL-6, IL-10
MDSCs	TGF-ß, IL-6, IL-10, IL-1ß
Tumor-associated neutrophils	VEGF, IL-1ß, IL-17
Burse dependent lymphocyte	IFN-γ, IL-4, IL-10, Antibody
Thymus dependent lymphocyte	TNF-α, IFN-γ, IL-2, IL-4

2.3. Classification of Immunotherapy

Immunotherapy aims at overcoming the immune suppression of tumors, enhancing the immunogenicity of TAAs, restoring their specific anti-tumor response and killing cancer cells to inhibit the occurrence and development of the tumor, and achieving the goal of eliminating the tumor finally.

There are two main categories of immunotherapy, the passive and the active. Passive treatment refers to the injection of exogenous immune effector substances into the body, such as genetic engineering antibody drugs which leads to the combination between antibodies and substances that directly kill tumor cells, including toxins, chemotherapy drugs, radionuclides. Adoptive T-cell therapy (ACT) is to inject immune effector cells into the body, such as DCs and cytokine-induced killer cells. Immune checkpoint inhibitors and tumor therapeutic vaccines are examples of active treatments to reactivate the immune system inhibited by tumor cells and stimulate the anti-tumor response.

3. Adoptive T Cell Therapy

3.1. Mechanism of adoptive T cell therapy

ACT is to collect autologous T cells extracted from peripheral blood, activate, genetically modify, and massively amplify them in vitro, and then inject them back intravenously to enhance their killing effect against tumor cells. There are many kinds of treatment according to the type of immune cells extracted from the patients, such as chimeric antigen receptor T cell (CAR-T) therapy. T cells amplify in vitro and utilize genetic engineering techniques to express CAR on the cell membrane that recognizes TAAs. The gene encoding chimeric antigen receptor is fused to the T cell activation fragment by genetic engineering techniques and transfects the extracted T cells by the transduction of lentivirus or retrovirus to enable T cells to specifically recognize and stimulate an anti-tumor response.

3.2. Application of CAR-T cell therapy

CAR is conducive to the direct recognition of TAAs without the restriction of human leukocyte antigens (HLA), which provides a more targeted therapy. Tisagenlecleucel is the first approved therapy choosing CD19 as the target and has been clinically used for relapsed or refractory acute lymphoblastic leukemia (ALL). These cells specifically bind to CD19 to induce the killing effect against tumor cells. Since CD19 is the unique marker of B lymphocytes, it effectively limits off-target toxicity and is nonlethal to B cells, leading to strong efficacy.

As the therapy matures with effectiveness in many cancers, researchers started to access its effect on patients with advanced PC. Raj et al. [10] conducted research into CEACAM7, a possible therapeutic target for PC, to analyze the feasibility of CAR-T cells using in vitro and in vivo models. A single dose of 2869 CEACAM7-targeted CAR-T cells was injected into five mouse models of advanced PC, and the tumors in three of them are entirely regressed. Since animal models cannot fully simulate the immunosuppression of TME in humans, drug therapy targeting TME should be combined in clinical practice. He et al. [11] engineered mesothelin-targeting(meso) CAR-T cells through the plasmid electroporation technique to express mesothelin for specific targeting of PC cells. In both in vitro and mice models, mesoCAR-T cells attacked PC cells continuously as expected, cleared tumor cells successfully, and eventually differentiated into memory T cells with little damage to major organs. Some other clinical advance is listed in Table 2.

Table 2. Clinical progress of CAR-T in patients with PC.

Therapy	Phase trial
Mesothelin-Specific CAR-T cells	I
CEACAM5-specific CAR-T cells	I

Because of low invasion of T cells, lack of targeted TME, and unstable expression after injection, CAR-T cells are hindered from infiltrating into the tumor site. A simple and efficient CAR-T delivery method is developed recently by adding CAR-T cells and cytokines into the hydrogel to improve the curative effect on solid tumors[12]. At present, other challenges for CAR-T therapy contain the absence of sustained attack and the evolution of CD19- tumor cells which cannot be recognized. Since biomarkers vary according to different stages of immunotherapy, diverse biomarkers to assess CAR-T cell efficacy and depletion after injection. Furthermore, current CAR-T cell therapy is based on autologous T cells which is time-consuming and inefficient. One opportunity to ameliorate these problems is the utilization of allogeneic CAR-T cells by donators which benefits patients in need of urgent treatment. Dual-targeted or tandem CARs consist of the co-expression of two separate CARs in each T cell, which recognize two distinct antigens is another possible way to optimize this technique.

4. Immune Checkpoint Inhibitor

4.1. Mechanism of immune checkpoint inhibitor

Immune checkpoints are defined as tumor cell surface antigens and immune cell surface receptors. Immune checkpoint inhibitors (ICIs), the monoclonal antibody drugs, specifically bind to antigens and receptors of specific immune checkpoints and inhibit the suppressive influence of tumor cells on immunocytes.

Cytotoxic T Lymphocyte associated antigen-4 (CTLA-4) is located in the cytoplasm of primary T cells with its B7 ligand on the cytomembrane of antigen-presenting cells (APCs). CTLA-4 will transfer to the cytomembrane through exosomes when the stimulatory signal is induced and the primary T cell is activated due to the binding of the B7 ligand to the CD-28. Then CTLA-4 can competitively combine with B7 due to a much stronger affinity, inhibiting T cell activation at the initial stage.

Programmed cell death protein 1 (PD-1) is low expressed in T cells normally, but overexpressed when T cells are exposed to antigenic stimuli chronically. Furthermore, activated T cells can induce high expression of PD-L1 in other cells by releasing cytokines such as TNF-γ and interleukin. The binding of PD-1 and PD-L1 inhibits T cells activation and even mediates apoptosis, as shown in Fig.2.

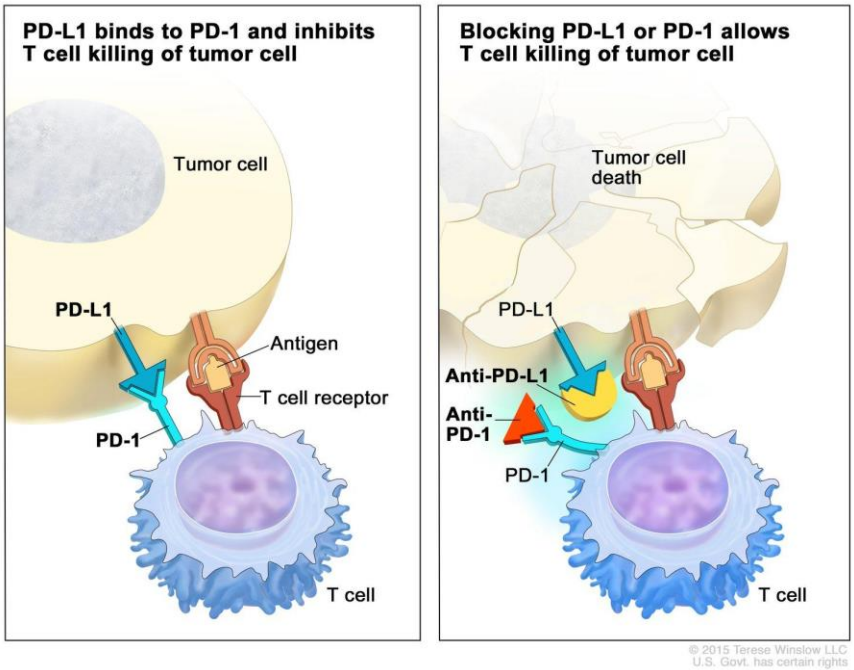


Figure 2. Mechanism of ICIs, taking PD-L1 and PD-1 as examples. [13]

4.2. Application of immune checkpoint inhibitor

Ipilimumab, a CTLA-4 inhibitor, is approved to have clinical value in metastatic melanoma treatment. Hodi et al. [14] reported Ipilimumab and a combination of Ipilimumab and a gp100 peptide vaccination have longer overall survival than gp100 alone in 676 patients with unresectable stage III or IV melanoma. Patients received Ipilimumab plus gp100, Ipilimumab alone and gp100 alone are analyzed with the respective median overall survival of 10.0, 10.1, and 6.4 months. The phase III clinical trials Check Mate 017 [15] and 057 [16] respectively reported that a PD-1 inhibitor Nivolumab was significantly more effective than docetaxel in squamous and non-squamous NSCLC. Overexpressed immune checkpoint VISTA in CD68 macrophages, an immune cell infiltrating the stromal region of PC, provided a potential immunotherapeutic target for PC. It is found that the low reactivity of PD-1/PD-L1 inhibitors to PC attributes to the immunosuppressive effect of the VISTA pathway on T cells among three patients with metastatic PC[17]. Therapies targeting different immune checkpoints made clinical progress as shown in Table 3.

Table 3. Clinical progress of ICIs in patients with PC. [18, 19]

Target	Therapy	Phase trial
Anti-CTLA4	Ipilimumab	Ⅱ
	Ipilimumab±GVAX	Ⅰ
	Tremelimumab (CP-675,206) + Gemcitabine	Ⅰ
	Tremelimumab±Durvalumab	Ⅱ
Anti-PD-1	Nivolumab + Mogamulizumab	Ⅰ
	Pembrolizumab (MK-3475)	Ⅰ
	Pembrolizumab + Gemcitabine + nab-Paclitaxel	Ⅰb/Ⅱ
Anti-PD-L1	Anti-PD-L1 Antibody	Ⅰ
	Atezolizumab	Ⅰ
	Atezolizumab + Navoximod (GDC-0919)	Ⅰ
	Durvalumab + Ibrutinib	Ⅰb/Ⅱ

In addition to the immune checkpoints mentioned above, more checkpoints have been discovered in the last few years. It is reported that a combination of several antibodies can achieve a better therapeutic effect than single inhibitor. Since immunoregulation is not simply linear regulation, the

interaction of signaling pathways as well as the influence of distinct TME plays an important role in the efficacy of ICIs. The existence of TAMs and MDSCs in the immunosuppressive TME obstructs the proliferation and response of normal effector T cells in pancreatic tissues, restricting the application of ICIs. Furthermore, the reason why resistance to the drugs is developed may be the defects in antigen presentation for the loss of MHC, the blockade of IFN-γ signaling pathways and so on. Realizing the unobvious effect of single ICI therapy in PC treatment, researchers combined them with other immunotherapy or traditional treatments like chemotherapy and radiotherapy to conduct more research.

5. Tumor vaccine

5.1. Mechanism of tumor vaccine

Tumors have developed a variety of methods to avoid immune detection. For example, when a specific immune checkpoint inhibitor was designed to block the connection of relevant immune checkpoints, there came another site to perform similar functions. There are two ways for tumor vaccines to restore the anti-tumor effect of the immune system. One is to enhance the immunogenicity of tumor cells when they do not express or reduce the expression of surface antigens, such as the nucleic acid vaccine, also called genetic vaccine, which is delivered to the patient by intramuscular injection and encodes antigens inserted by genetic engineering technique. The other is to supplement some lacking costimulatory molecules and adhesion factors, such as polypeptide vaccine which delivers tumor antigen peptide onto the surface of T cells to express TAAs and induce cytotoxic T cell response.

5.2. Whole- cell vaccine

Whole- cell vaccines contain a full range of TAAs, reducing the steps required to pre-identify a target and eliciting a full anti-tumor response. Tumor cells are usually modified to enhance their immunogenicity with the expression products of foreign genes directly acting on immune cells to promote their proliferation and differentiation, such as various cytokines and chemokines.

Take the GVAX vaccine as an example, granulocyte-macrophage colony-stimulating factor (GM-CSF) is secreted after injection to promote antigen presentation function of B and T cells and stimulate the anti-tumor response. Although GVAX vaccine had a positive effect in mice models, the effect in clinical trials of PC treatments was limited. In a phase II study, Wu et al. [20] found some positive changes in the number of macrophages in the TME of the GVAX vaccine and Ipilimumab maintenance therapy for PC, the survival rate was not improved in line with the expectation though.

5.3. Dendritic cell vaccine

DCs are the most powerful APCs in the human body and are responsible for the activation of cell-mediated immunity. After specific recognition and binding between the receptors on the dendritic cell membrane and the ligands on the T cell membrane, a large number of cytokines can be generated by DCs to induce the proliferation and differentiation of initial T cells. Chemokines are secreted for the specific chemotaxis of primary T cells, enhancement of T cell activation and initiation of the immune response. Moreover, DCs participate in the maturation and differentiation of B cells, promoting B cells to secrete a large amount of immunoglobulin IgM and IgA and inducing the differentiation of primary and memory B cells into plasma cells to exert the anti-tumor efficiency by secreting interferon I [21].

DC vaccine therapy is to combine DCs extracted from patients with a large number of TAAs in vitro, and then inject them back into patients after culture, maturation, and activation of the modified DCs. Also, injecting DCs expressing tumor antigens, cytokines or chemokines can enable them to express stably and chronically.

5.4. Nucleic acid vaccine

Nucleic acid vaccine, also called genetic vaccine, inserts some gene fragments to encode antigens into the eukaryotic expression plasmid and inject into the patient through gene gun injection, intramuscular injection, and liposome encapsulation, enabling exogenous antigen genes continuously to express in vivo. This therapy is still placed in the experiment and theories research stage without enough valuable clinical data, but there is still large space for further research and application prospect.

Although various tumor vaccines have been under elaborative research with some of the clinical progress shown in Table 4, the clinical effects are not promising. As the most efficient vaccine from preparation to clinical application, the peptide vaccine is underperforming in expressing enough immunogenicity. The preparation of the DC vaccine is complicated and requires personalized design, which is not conducive to popularization. Theoretically, the nucleic acid vaccine can induce both humoral and cellular immunity with easy preparation. However, these three methods mentioned above saw no ideal effectiveness. How to improve vaccine delivery systems for a quicker and more effective response is one of the great challenges.

Table 4. Clinical progress of tumor vaccines in patients with PC. [18, 19]

Type	Therapy	Phase trial
Whole-cell vaccine	GVAX	Ⅱ
	GVAX /Cyclophosphamide + CRS-207	Ⅱb
	ANZ-100+CRS-207	Ⅰ
	Algenpantucel-L	Ⅲ
Peptide vaccine	KRAS peptide + GVAX	Ⅰ/Ⅱ
	TG01/GM-CSF + Gemcitabine	Ⅰ/Ⅱ
	21-mer peptide	
	Telomerase peptide (GV1001)	Ⅰ/Ⅱ
	MUC-1 peptide	Ⅰ/Ⅱ
	MUC1 peptide with SB-AS2 adjuvant	Ⅰ
	G17DT	Ⅱ
Dendritic cell vaccine	MUC1 peptide-pulsed autologous DC vaccine	Ⅰ/Ⅱ
	WT1-pulsed DC vaccine with Gemcitabine	Ⅰ
	Peptide-pulsed DCs with poly-ICLC	

6. Oncolytic Virus

6.1. Antitumor mechanisms of oncolytic virus

Oncolytic viruses (OVs) can recognize and specifically bind TAAs, selectively attack tumor cells, and self-replicate without interfering with the survival of other normal cells. OVs proliferation leads to necrosis of tumor cells and progeny viruses release to infect uninfected tumor cells. The tumor antigen is released and presented to effector T cells after the lysis of tumor cells, activating immune response. In addition, OVs specifically infect tumor-depended blood vessels and trigger an inflammatory response that mediates the formation of microthrombus in tumor blood vessels, blocks nutrient transport, and promotes tumor cell necrosis [22]. Oncolytic virus therapy includes natural strains and genetically modified strains and there are three aspects of gene modification. One is to enhance tumor targeting. Although some strains are targeted to tumors naturally, such as reovirus and autonomous parvoviruses H-1, others like adenoviruses and herpes simplex viruses (HSV) require gene modification by inserting genes for binding proteins targeted by tumor cells to enhance tumor-targeting. Two is to reduce the lethality of oncolytic viruses and improve their safety by knocking out key virulence genes. Three is to insert therapeutic genes, such as pro-apoptotic genes, oncogenic factor knockout genes that can kill tumor cells or genes expressing interferon and interleukin to promote immune responses [23].

6.2. Classification and application of oncolytic virus

There are two groups of OVs according to their natural targeting of tumors. One is natural tumor-targeting strains, such as reovirus, and the other is genetically modified strains, such as HSV and adenovirus.

Reovirus is a natural virus with double-stranded RNA. In normal cells, its gene expression product activates protein kinase R(PKR), phosphorylating eukaryotic translation initiation factor (EIF2α), inhibiting intracellular mRNA translation, and preventing the virus from replication. While in the cells of PC with RAS pathway activation, the PKR signal transduction pathway is inhibited and a large number of reoviruses replicate in tumor cells, leading to cell lysis.

As a large enveloped virus, HSV has large genome and strong replication ability which bring great potential for gene modification. For example, the US3 gene encodes a multifunctional protein kinase with the capability of inhibiting apoptosis. L1BR1, an HSV-2 mutant with US3 deletion, can replicate in PC cells, cause oncolysis and suppress tumor growth [24].

Adenovirus is a small non-enveloped virus which can specifically bind to coxsackie/adenovirus receptors on the surface of cytomembrane. Its protein outer shell is degraded through internalization of binding between viral pentosan and integrin and its genome inserted into the host for transcription expresses the E1A protein that targets to kill PC cells [25]. The modified adenovirus LOAd703 replicates specifically and intracellularly Under the cooperative action of free transcription factors. The free release of eukaryotic transcription factors E2F is closely related to the phosphorylation of retinoblastoma protein (Rb). Most PC cells can be targeted by LOAd703 due to excessive phosphorylation of Rb protein [26].

In a phase Ib study, ten PC patients received Pelareorep and Pembrolizumab in combination with some chemotherapeutic drugs and presented promising efficacy eventually. Three of the ten patients achieved durative effect with one responding for 17.4 months and the other two remitting for 4 and 9 months respectively [27].

Compared with two mutant viruses (R3616 and hrR3), Kasuya et al. [24] evaluated the efficacy of a novel mutant virus, US3 locus-deficient HSV L1BR1, under which treatment visible tumors in two mice models disappeared in 4 weeks among the total ten, providing a novel encouraging oncolytic virus therapy.

7. Conclusions

Due to the hidden clinical symptoms, the early diagnosis rate is low, most PC patients are at an advanced stage once diagnosed. Moreover, the low probability of surgical resection and poor prognosis after surgery are obstacles to the treatment of PC. Chemotherapy and radiotherapy are both common treatments of cancer, but the effect in the treatment of PC is not obvious.

With the emergence of immune checkpoint inhibitor, tumor vaccine, adoptive T cell therapy, and oncolytic virus, immunotherapy has become the hotspot in expectation of improving the prognosis of patients with PC and reducing the death rate. However, due to the low invasion of solid tumor immune cells, the dense stromal barrier of PC, and the immunosuppressive TME, the application of immunotherapy in the treatment of PC cannot be as successful as those in hematologic tumor treatment. At present, both single immunotherapy and multiple immunotherapy combinations have not achieved the expected efficacy. Therefore, researchers have turned their attention to the combination of immunotherapy with traditional therapy, such as surgery, chemotherapy, and radiotherapy which has been proved promising.

With the development of the High-throughput sequencing technique, the whole-genome analysis showed significant heterogeneity among different PC patients. Big data monitoring methods based on genomics, transcriptomics, proteomics, and metabolomics analysis will provide precise and personalized drug targets for patients with PC. In addition, since single immunotherapy saw dissatisfactory progress, personalized combination therapy will be universal in the future. It is full of challenges to treat PC within tight time constraints considering that early diagnosis is extremely

difficult because of the lack of effective tumor markers and precision therapy is necessary for patients with significant individual differences. To sum up, there are many tasks to overcome, such as how to identify tumor markers with higher specificity and sensitivity, overcome the complex microenvironment of immune tolerance of solid tumors, simplify the process of individual tumor gene detection and improve the timeliness and accuracy of developing treatment strategies.

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