1. Introduction

Pancreatic cancer is an aggressive and highly lethal malignant disease. It is the fourth most common cause of cancer deaths in the United States. Recent cancer statistics from national cancer institute showed that overall 5-year relative survival for 2002–2008 was 5.8%. (23.3% for localized disease, 8.9% for pancreatic cancer with regional dissemination and 1.8% with distant metastasis) [1]. Unfortunately, due to the late onset of symptoms, more than 80% of patients with pancreatic cancer are diagnosed with locally advanced or metastatic disease with very poor prognosis and are not eligible for surgery which is the only curable therapy [2].

Radical surgery remains the only chance for long-term survival. In general, tumor with metastatic lesion excludes patients from curative-intended surgery [3]. Patients with unresectable pancreatic cancer without disease progression after chemo/radiotherapy should be considered for radical surgery [4]. A recent meta-analysis results showed that palliative resections (R2 resection or tumor debulking) in pancreatic cancer are associated with increased morbidity, mortality, and hospital stays compared with bypass procedures, while the survival benefit is very modest, therefore, planned tumor debulking is not recommended [5]. Even when surgery can be performed, prognosis remains very poor due to the high propensity of the tumor for local regional recurrence and overall 5-year survival rate after curative resection still remains less than 20% [6,7]. Nearly all survivors are in the early stage disease at the time of diagnosis, suggesting that early detection or effective neo-adjuvant approaches are of great importance to improve the dismal outcome of pancreatic cancer.

It is clear now that even patients with potentially resectable pancreatic cancer require multimodality treatments including chemotherapy and/or radiation to improve resectability and reduce recurrence. The most widely used chemotherapy for patients with advanced pancreatic cancer, as well as patients requiring adjuvant therapy after surgery, is gemcitabine or gemcitabine-based chemotherapy. All the current chemotherapies for pancreatic cancer are associated with dose-limiting hematologic toxicity and other adverse effects that require ongoing monitoring and dosage adjustment to balance the benefits and risks of therapy [8].

Radiotherapy has a role in the adjuvant management of pancreatic cancer [9]. External beam radiotherapy (EBRT) is used for curative purposes as adjuvant treatment to surgery, and for palliative purposes in cases where surgical resection is not feasible. Nevertheless, the effects of EBRT following pancreatectomy for pancreatic cancer remain controversial. Intraoperative radiotherapy (IORT) is developed to optimize radiation deposit precision within the target volume (defined intra-surgically) and protection of normal tissues adjacent to the target [10]. IORT has been considered for pancreatic cancer therapy since local recurrence rates are very high. It reaches pain palliation in most cases [11]. IORT could slightly increase survival among pancreatic cancer patients in localized stages. However, the results were not conclusively in favor of IORT in the case of pancreatic cancer in locally advanced and metastatic stages [12,13].

Despite improvements in short-term surgical outcomes, the use of newer chemotherapeutic agents, development of targeted
agents and more precise delivery of radiation, the 5-year survival rates for early-stage patients remains less than 25% [14]. Pancreatic cancer is one of the cancers for which survival has not been substantially improved during the past 30 years. Thus, it is clear that novel effective therapeutic approaches for pancreatic cancer are urgently needed. The rapid development of modern molecular biology uncovered the genetic mechanisms controlling pancreatic carcinogenesis, which hopefully will improve the outcome of pancreatic cancer patients in the future. Viral therapy has been regarded as a potential new treatment modality for many years because of its specificity and high potency. The aim of this review is to summarize what has been accomplished in the field of viral therapy for pancreatic cancer.

2. Viral therapy for pancreatic cancer

Oncolytic viruses, genetically programmed to replicate within tumor cells but not in normal cells, directly induce cytotoxic effects via cell lysis, are currently being explored in preclinical and clinical studies of various cancers such as head and neck cancer, pancreatic cancer, ovarian cancer, prostate cancer and malignant glioma [15]. Moreover, oncolytic viruses produced from initially infected cancer cells can spread to surrounding cancer cells thereby can enhance the therapeutic effects. The efficacy of oncolytic viruses depends on multiple actions including direct tumor lysis, modulation of tumor perfusion and stimulation of tumor-directed immune responses (Fig. 1). Oncolytic viruses can be modified in a variety of ways to improve their selectivity including: (1) by deleting viral genes necessary for efficient replication in normal cells but not tumor cells; (2) by regulating the transcription of viral replication proteins through the use of exogenous, tissue specific promoters (i.e. transcripcional targeting), or (3) by retargeting viral infection specifically to tumor cells (i.e. transductional targeting). The variety of oncolytic viruses such as adenovirus, herpes simplex virus, influenza virus, Newcastle disease virus, poliovirus, reovirus, vaccinia virus and vesicular virus have been developed for cancer treatment [16–19].

2.1. Oncolytic adenovirus

Adenovirus (Ad) is one of the most commonly used vectors for gene therapy and two products of adenovirus have already been approved for treatment of cancer in China (Gendicine(R) and Oncorine(R)) [20]. Replication-selective oncolytic adenoviruses are designed especially for replication restricted to cancer cells. Infected cells can be killed by several mechanisms including direct lysis, expression of toxic proteins, induction of inflammatory cytokines and T-cell mediated immunity (Fig. 1).

2.1.1. Adenovirus developed by gene modification

ONYX-015 (dl1520) is the first oncolytic Ad used to treat human cancers. It is an E1B gene (a gene that inhibits the function of the
tumor suppressor gene p53)-deleted adenovirus that replicates in and selectively lyses cancer cells, have previously reported antitumoral efficacy and increased survival following intratumoral injection in human tumor xenografts [21]. In the clinical trial, after ONYX-015 endoscopic ultrasound (EUS) guided intratumoral injection in combination of intravenous gemcitabine therapy, 2 patients had partial regressions, 2 had minor responses, 6 had stable disease, and 11 had progressive disease or had to go off study because of treatment toxicity (Table 1). It indicates that intratumoral injection of ONYX-015 via EUS to treat pancreatic cancer is feasible and well tolerated either alone or in combination with gemcitabine [22]. H101 (also named Oncorine(R)) is a similar oncolytic adenovirus with E1B-55KD and partial E3 deleted and it is the first world commercially approved oncolytic viral agent for cancer. Clinical data for treating head and neck cancers show that H101 is well tolerated either alone or in combination with chemotherapy (Table 1) [23,24].

A replication-selective mutant (AdΔΔ, E1B19K and CR2 deleted) was generated targeting alterations in pRB (ΔCR2) and apoptosis pathways (ΔE1B19K) with intact E3 region [25]. It improved efficacy and selectivity both as a single agent and in combination with standard chemotherapy in prostate and pancreatic cancer. A double-deleted AdDeltaDelta mutant (deleted in the pRB-binding E1A/CR2 region and E1B19K) was reported to selectively replicate and enhance cell killing in combination with DNA-damaging cytotoxic drugs in pancreatic cancer cells in vitro and significantly prolong time to tumor progression in two human pancreatic tumor xenograft models [26]. This indicates that AdDeltaDelta has low toxicity to normal cells while potently sensitizing pancreatic cancer cells to DNA-damaging drugs, and holds promise as an improved therapeutic strategy for pancreatic cancer.

2.1.2. Adenovirus armed with therapeutic genes

There are still several disadvantages of conventional oncolytic adenovirus therapy for cancer including limited infection of cancer cells, limited intratumoral spread and limited specificity for cancer cells as well. A number of studies were conducted focusing on increasing treatment efficacy as well as high selectivity for cancer cells [27]. Then various adenoviruses were further engineered to express tumor-associated antigens, cytokines, chemokines, or other immunomodulatory elements, which have been shown to induce antigen-specific effector, resulting both in full therapeutic cures and even induction of life-long tumor immunity in animal models [20].

We recently developed a human carinoembryonic antigen (CEA) promoter-regulated oncolytic adenovirus carrying the Hsp70 gene (AdCEAp-Hsp70). It significantly increased the expression levels of Hsp70 in the CEA-positive pancreatic cancer cells, resulting in an overall reduction in the survival of cancer cells in vitro. Additionally, therapeutic effect was detected in the virus treated animal models [28]. In order to efficiently infect and lyse pancreatic tumors, the knob domain of the Ad serotype 5 was modified with a serotype 3 knob domain and the CXCR4 promoter was incorporated to regulate Ad E1A gene expression (Ad5/3-CXCR4-E1A). Compared with an unmodified virus (Ad5-CXCR4-E1A), Ad5/3-CXCR4-E1A was most efficient in infecting and lysing pancreatic cancer cells. Smaller tumor size, greater body condition scale score and longer survival time were detected in the Ad5/3-CXCR4-E1A group [29].

Combination therapy with interferon alpha (IFN-α) is correlated with improved survival in patients with pancreatic cancer. A novel tumor-specific conditionally replicative IFN-expressing adenovirus was shown to posses the potential to locally deliver IFN and avoid systemic toxicity resulting in stronger tumor suppression when compared with non-replicating IFN-expressing vectors [30]. A similar cyclooxygenase-2-targeted, IFN-expressing, conditionally replicative adenovirus was also demonstrated highly favorable effects in vitro and in vivo for pancreatic cancer therapy. These data suggested that adenovirus-based IFN therapy offers a new treatment opportunity for pancreatic cancer [31].

2.1.3. Other adenovirus

RNA interference is an emerging technique used to downregulate gene expression by sequence-specific post-transcriptional targeting. As oncolytic adenoviruses replicate specifically in cancer cells and continuously express shRNA, resulting in long-term expression of therapeutic siRNA molecules as well as avoiding non-specific shRNA expression in normal cells. The application of siRNA technology in oncolytic viruses may be particularly effective [32]. A replication-competent, oncolytic adenovirus, ONYX-411, was used to deliver a mutant K-ras siRNA transgene to human pancreatic cancer cells in a recent study [33]. The findings of the study indicated that Internavac or interfering RNA vector could generate a two-pronged attack on tumor cells through oncogene knockdown and viral oncolysis, resulting in a significantly enhanced antitumor outcome.

Until now, adenovirus serotype 5 is most commonly used to generate oncolytic Ad. However, viral replication in hepatocytes could result in severe liver toxicity, thus limits its application in metastatic disease. Moreover, rapid clearance of virons injected intravenously further impairs the anticancer efficacy. Adenovirus 6 (Ad6) was reported to have less liver toxicity and escape Kupffer cells absorption after systemic administration. A novel oncolytic Ad6imiK was generated based on Ad6 recently to reduce hepatotoxicity. A liver-specific microRNA miR122 were incorporated into E1A gene of Ad6. The negative regulation of miR122 significantly

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**Table 1** Viruses applied in clinical trials.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutations</th>
<th>Trial details</th>
<th>Outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONYX-015 (d1520)</td>
<td>E1B deleted Ad E1B-55KD and partial E3 deleted Ad</td>
<td>Phase I/II trial in PC Phase I-III in head, neck cancers; in PC not completed</td>
<td>Feasible and well tolerated Well tolerated and 79% response rate for H101 plus chemotherapy</td>
<td>Hecht [22]</td>
</tr>
<tr>
<td>H101 (Oncorine(R))</td>
<td>J5-1 strain of HSV-1 with GM-CSF and deletion of both IFP34.5 and ICP47</td>
<td>Phase I (phase II in solid tumors, phase III in melanoma, phase I in PC is ongoing</td>
<td>Well tolerated at high and repeated doses</td>
<td>Yu From 2000 to 2004</td>
</tr>
<tr>
<td>OncoXVCA-C5F</td>
<td>Naturally HSV-1 mutant Wild-type revovirus type 3 Dearing</td>
<td>Phase I in advanced PC Phase I in solid tumors; phase II in metastatic melanoma; phase II in PC</td>
<td>Safe and feasible 37% patients with tumor response in phase I; 75–90% with tumor necrosis in phase II</td>
<td>Hu [41], Harrington [42], Kaufman [64,65], Nakao [69]</td>
</tr>
<tr>
<td>Reoysin®</td>
<td>(CEA) promoter-regulated oncolytic adenovirus carrying the (CEA) promoter-regulated oncolytic adenovirus carrying the</td>
<td></td>
<td>Recruiting (NCT01280058) Significant increase in OS</td>
<td>Morris [74], Galanis [73], Maitra [70], Kaufman [80]</td>
</tr>
<tr>
<td>VACA</td>
<td>VACA expressing CEA, MUC-1, B7.1, ICAM-1 and LFA-3</td>
<td>Phase I in advanced PC</td>
<td></td>
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The majority of clinical trials involving oncolytic virus therapies targeting solid cancers including pancreatic cancer have shown to be safe and feasible. Abbreviations: PC: Pancreatic cancer; GEM: Gemcitabine; OS: Overall survival.
decreased Ad6 replication in hepatocytes and improves its safety profile, which allows increasing therapeutic doses leading to improved anticancer efficacy of systemic treatment [34].

2.2. Oncolytic herpes simplex virus

Herpes simplex viruses (HSV) including HSV-1 and HSV-2 are both large enveloped viruses with double stranded DNA. HSV-1 infection often causes a self-limiting disease with local lesions like sores in mouth and lips and persistence in neural ganglia. HSV has been genetically engineered to conditionally replicate only in cancer cells. It has been shown to be a promising therapeutic agent for cancer treatment (Fig. 1). The first replication-competent oncolytic HSV (a thymidine kinase-negative mutant of HSV-1) was reported for the therapy of human malignant gliomas in 1991 [35]. Then a number of studies have been conducted focusing on the unique features of HSV for cancer therapy.

2.2.1. Distinct advantages and disadvantages of oncolytic HSV

Genetically engineered conditionally replicating HSV seems to be promising for cancer therapy because it carries several special characteristics when compared with other oncolytic viruses. (1) There are several available anti-HSV specific medications such as acyclovir (ACV) and ganciclovir (GCV). When viral therapy causes an unexpected infection, it can be used to control adverse effects which makes it to be obviously an advantage over all the other oncolytic viruses; (2) As compared with measles virus, the large size genome (152 kb) of HSV contains many nonessential genes which can be mutated or replaced with large transgenes for therapeutic purposes; (3) With wide cell tropism, HSV can infect various tumor cell types and total cell killing can be achieved with a relatively low multiplicity of infection. As a consequence, oncolytic HSV could be potentially used in many types of cancer including brain cancer, breast cancer, gastric cancer, prostate cancer, pancreatic cancer, etc. [36–40]; (4) HSV normally does not integrate into cellular DNA, thus during oncolytic HSV treatment, the risk of insertion of foreign genes into the host seems minimal. Genetically engineered HSV has confirmed to be safe in vivo even in human without severe side effects [41,42]; (5) Compared with other viruses, HSV typically can infect and kill target tumor cells more rapidly. Rapid replication may be important for the oncolytic treatment because the immune system of host may be more likely to restrict the spread of slower growing viruses.

There appears to be some disadvantages of HSV as a therapeutic agent for cancer. As HSV is a common human pathogen, a lot of people carry the anti-HSV antibodies, which theoretically may neutralize the oncolytic HSV and reduce the therapeutic potency. However, studies showed that the pre-existence of the anti-HSV immunity in different mouse models did not affect the cell-to-cell spread of the virus and did not have significant influence on the treatment effect [43,44].

2.2.2. Oncolytic HSVs modified with gene deletion for conditionally replication

The most widely used strategy to genetically engineer oncolytic HSV is to restrict replication of HSV only in cancer cells, including deletion of essential genes for viral replication and deletion of genes that counteract the IFN-related protein kinase (PKR) response. Deletion of γ1 34.5 gene (R3616, HSV1716) or ICP6 gene are good examples. Inactivating these genes enables the virus to selectively replicate in the dividing cells, whereas sparing the normal nondividing cells. The attenuation of the γ1 34.5 gene deletion mutants is related to the cellular PKR response. The activity of PKR in cancer cells affects the virus replication significantly. Cancer cells with higher mitogen activated protein kinase (MAPK, an inhibitor of PKR) activity are highly susceptible to γ1 34.5 mutants [45,46]. As a consequence, the heterogeneity of MAPK status of cancer cells will limit the method of deletion of γ1 34.5 gene. The activation of the K-Ras pathway could lead to inhibition of PKR activity [47]. Interestingly, oncolysis of pancreatic cancer cells by γ34.5 deletion mutants R3616 is neither associated with defective PKR activity nor with enhanced Ras signaling. By contrast, it is associated with dysregulation of the PI3K pathway [48].

HSV-1 based mutant hrR3 lacks the UL39 gene encoding the ICP6 protein. Lack of the ICP6 protein causes the virus conditionally replicate in cancer cells. hrR3 possesses an intact HSV thymidine kinase (HSV-tk) gene that can be used for metabolic activation of GCV, which acts to disrupt cellular and viral DNA replication. Long-term survival was achieved in 70% of pancreatic cancer mice treated with intraperitoneal injection of hrR3 followed by systemic GCV treatment, while in 40% of mice with injection of hrR3 only and 0% in untreated mice [49]. R3616 is a conditionally replicating HSV-1 mutant lacking the γ34.5 gene encoding ICP34.5 protein. Replication of R3616 is severely restricted in normal cells, because the expression of ICP34.5 in normal cells prevents a protein shutoff mechanism that is associated with eIF2α dephosphorylation. Most cancer cells lack the normal protein shutoff mechanism so that viral replication can proceed [50]. A recent study compared the efficacy of hrR3 or R3616 plus gemcitabine against pancreatic cancer with peritoneal dissemination [51]. Long-term survival was observed in 60% of mice treated with an intraperitoneal injection of R3616 followed by gemcitabine, 50% in R3616 alone group, 30%, 20% and 10% in hrR3, in hrR3 followed by gemcitabine and in gemcitabine alone group respectively. Combination of gemcitabine with R3616 was shown to be more effective than combination of gemcitabine with hrR3. Interestingly, mice treated with hrR3 followed by gemcitabine showed a lower LTS rate than those treated with hrR3 alone. A similar study of L1BR1 (US3 locus-deficient HSV-2) in combination with 5FU and cisplatin for pancreatic cancer therapy showed that the combined treatment with L1BR1 and these anticancer drugs enhanced apoptosis significantly. Compared with R3616 and hrR3, L1BR1 showed the lowest replication capacity in normal human hepatocytes, but the highest tumor-reducing effect in vivo [52]. Chemotherapy drugs potentially con-note to inhibit oncolytic virus replication to some degree, but this may be influenced by the differences in the characteristics of each virus caused by gene mutation. Therefore, the characteristics of each virus should be considered carefully to determine if they are suitable for use with the chemotherapeutic drugs chosen.

G207 was constructed from HSV-1 strain F with both copies of γ134.5 deleted and the UL39 gene (also named ICP6 gene) inactivated by insertion of the Escherichia coli LacZ gene [53,54]. It was effective against a wide variety of solid tumors including pancreatic cancer [53,55,56]. NV1020 has one copy of the γ134.5 gene deleted with ICP6 gene intact. The administration of NV1020 may lead to a higher effective dose at the tumor site, since its relatively higher proliferative rate than G207 in pancreatic cancer cells [57]. NV1023, a derivative of NV1020, could effectively treat pancreatic cancers with neural invasion and preserve neural function [58]. The combination of NV1023 and radiation yielded a synergistic oncolytic effect in various pancreatic cancer cell lines which was mediated by a substantial increase in apoptosis [59]. The combination of NV1066 (another derivative of NV1020) and hyperthermia significantly increased cell kill in pancreatic cancer in vitro. Hyperthermia enhanced NV1066 replication through a heat shock protein pathway [40].

Most of the current oncolytic HSVs are derived from HSV-1 by deletion of the essential genes required for replication. Besides L1BR1, FusOn-H2 is another oncolytic virus constructed from HSV-2 with the deletion of ICP10 gene encoding serine/threonine protein kinase activity. Intratumoral injection of FusOn-H2 completely eradicated s.c. pancreatic cancers in all animals. Systemic
injection of FusOn-H2 produced clear antitumor effects. Completely eradication of orthotopic tumors in 75% of the animals and completely prevention of local metastasis were observed when FusOn-H2 was given i.p. FusOn-H2 has shown to be a promising approach for treatment of pancreatic cancer [60].

2.2.3. Oncolytic HSVs focusing on improving the efficacy

One of the limits of oncolytic virus therapy is the potential of the treatment. Chemokines and cytokines can both support and control tumor growth. Even some cytokines can regulate the anti-tumor immune response, other cytokines can also modulate the response of tumor stroma and affect tumor angiogenesis [61]. Several strategies aimed at delivering or interfering with cytokine gene expression via oncolytic viral therapy had been utilized to enhance antitumor efficacy. This is another main type of strategy to construct oncolytic HSVs by focusing on boosting the local immune response. In this section we discuss some of the approaches used to modulate cytokine expression in conjunction with oncolytic viral therapy. Granulocyte–macrophage colony-stimulating factor (GM-CSF) is the immune gene inserted most successfully into oncolytic viruses. The preference for GM-CSF derives from its properties as a vaccine adjuvant, its potent ability to generate systemic adaptive antitumor immunity in vivo after expression in tumor cells, http://www.ncbi.nlm.nih.gov/ezproxyhost/library.tmc.edu/pmc/articles/PMC3129809/ which is associated with the recruitment and differentiation of activating dendritic cells (DC) in the tumor microenvironment [62,63].

JS1/34.5-47/GM-CSF (OncoVEXGM-CSF) is an oncolytic virus based on the JS-1 strain of HSV-1 engineered to express human GM-CSF. It has genetic deletions in ICP34.5, providing tumor-selective replication, and ICP47, which otherwise blocks antigen presentation to MHC class I and II molecules by inhibiting TAP1 and TAP2 transporters. The ICP47 deletion also increases the expression of US11, an inhibitor of PKR activation, promoting oncolytic selectivity. It contains the human GM-CSF coding sequence under the human cytomegalovirus immediate/early promoter [43]. OncoVEXGM-CSF mediates therapeutic activity through two distinct mechanisms: direct oncolytic destruction of tumor cells and induction of tumor-specific immunity through stimulation of DCs and presumably subsequent priming of antigen-specific T cell immunity. Local and distant antitumor immune responses have been observed in preclinical models and previous clinical studies. In the phase I and II study against solid tumors such as head and neck squamous cell cancer, breast cancer, gastrointestinal cancers, and malignant melanoma, OncoVEXGM-CSF was confirmed to be well tolerated at high and repeated doses [41,42]. Direct injection of OncoVEXGM-CSF induces local and systemic antigen-specific T cell responses and decreases T-reg, Ts, and Myeloid-derived suppressor cells (MDSC) in melanoma patients exhibiting therapeutic responses [64]. Followed the promising results of the phase II clinical study, the OncoVEXGM-CSF is now being tested in a phase III clinical trial in unresectable melanoma in several countries [65]. The phase I clinical study of intratumoral injection of OncoVEXGM-CSF into advanced pancreatic cancers through endoscopic ultrasound-guided fine needle is also ongoing [63]. Various immunomodulatory molecules including interleukin-4 (IL-4), IL-12 and interferon (IFN) have also been applied for expression by HSV to enhance antitumor efficacy [62,66].

In addition to the immunomodulatory molecules, there are other approaches including therapeutic genes introduced to enhance the oncolytic HSV therapeutic potency. A further virus based on OncoVEXGM-CSF backbone was developed through the combined expression of a highly potent prodrug activating gene [yeast cyto-sine deaminase/uracil phospho-ribosyltransferase fusion (Fcy::Fur)] and the fusogenic glycoprotein from gibbon ape leukemia virus (GALV). The results showed that GALV expression increased the tumor cell killing at least 30-fold in vitro and tumor shrinkage 5- to 10-fold in vivo, and additional expression of Fcy::Fur combined with 5-fluorocytosine administration improved tumor shrinkage further. Therefore, the combined expression of the GALV protein and Fcy::Fur provided a highly potent oncolytic virus with improved capabilities for local tumor control [67].

2.2.4. Oncolytic HSVs in clinical trials

HF10 oncolytic virus is a replication competent, naturally occurring HSV-1 mutant that can replicate and spread more efficiently than wild-type HSV-1 strains in most types of cancer cells [68]. In a phase I clinical trial of intraoperative intratumoral injection of HF10 in advanced pancreatic cancer, some therapeutic potential based on tumor marker levels, survival, pathological findings and diagnostic radiography was observed without any adverse side-effects. The tumors were classified as stable disease in 3 patients, partial response in 1 patient and progressive disease in 2 patients. It indicated that HF10 is a safe and feasible treatment with some therapeutic potential (Table 1). Because of the promising results, phase II and phase III study are in plan. The HF10 injection by using an endoscopic ultrasound for non-resectable pancreatic cancers is ongoing [69]. OncoVEXGM-CSF is another ongoing oncolytic virus delivered by endoscopic ultrasound needle for pancreatic cancer [63].

2.3. Other viruses

Reovirus is a nonenveloped double-stranded RNA virus and replication of it is related to high activity of oncogenic Ras. Incidence of K-ras mutation has been found to be very high in pancreatic cancer (85–90%), which makes it a promising candidate for pancreatic cancer treatment. The scientific rationale for its development as an anticancer agent stems from the fact that it preferentially replicates in and induces lyses of cells with an activated K-ras pathway [70]. In a liver metastasis from pancreatic cancer model, reovirus was delivered intraportally, decreased the number and size of treated tumors was observed without reovirus-related toxicities and deaths [71]. When oncolytic reovirus was delivered into peritoneal cavity of animal model with pancreatic cancer, the tumor volumes were significantly less that in the therapy group than in the control group. In addition, the amount of ascites was decreased in the therapy group. Immunohistochemical examination revealed that reovirus replicated only in the disseminated nodules but not in surrounding normal tissues. There were no serious side effects observed. These data suggested that intraperitoneal administration of reovirus might be an effective and safe therapeutic modality for pancreatic cancer [72]. Reoysin® is the trade name of the therapeutic version of human reovirus containing a purified isolate of 1 × 1011 TCID50 (tissue culture infective dose) and it has been used in many clinical trials including in pancreatic cancer in combination with Gemcitabine [70,73,74].

Parvoviruses such as parvovirus H-1 (H-1PV) may selectively infect and lyse cancer cells. It has been shown to possess not only direct oncolytic but also immunomodulating properties, serving as an adjuvant to prime the immune system to react against infected tumors [75]. Until now, parvoviruses have been successfully used for the experimental treatment on animal models of human glioma, neuroblastomas, lymphomas, pancreatic carcinomas and breast tumors [76]. The combination use of gemcitabine with H-1PV in vitro resulted in synergistic cytotoxic effects and with drug-resistant cells remaining sensitive to virus killing. Reduction of tumor growth, prolonged survival of the animals and absence of metastases on CT-scans were observed in vivo study. The data suggest that parvoviruses can be best combined with gemcitabine in a two-step protocol in addition to the potential use as monotherapy for pancreatic cancer [77]. A recent study showed that
IFN-γ could improve oncolytic H-1PV efficacy for the treatment of peritoneal carcinomatosis in pancreatic cancer [78]. Another study showed that parvovirus H-1PV infection of PDAC cells could increase NK cell capacity of NK cell-mediated tumor cell killing, suggesting the hope of a combinatorial therapeutic approach against PDAC [79]. They also found that parvoviruses armed with IL-2 or the chemokine MCP-3/CCL7 could lead to a strong antitumor response in PDAC tumors via activated NK and monocytic cells [80].

Measles virus (MV) vaccine strains have shown promising oncolytic activity against a variety of tumor entities. An engineered measles virus expressing the sodium-iodide symporter gene (MV-NIS) showed oncolytic activity in human pancreatic cancer xenografts with reduced tumor growth and increased survival in treated mice [81]. However, in the further combination therapy study, the synergy between MV-NIS-induced oncolysis and NIS-mediated [131I] radiotherapy in pancreatic cancer xenografts was not detected, which might be not due to a lack of radiosensitivity but rather to a nonuniform intratumoral distribution of MV-NIS infection [82]. Recently, a fully retargeted and armed MV-PNP-anti-PSCA was generated with prostate stem cell antigen (PSCA) expressed only on pancreatic adenocarcinoma. To enhance oncolytic efficacy, it has been modified to express suicide genes, the prodrug convertase purine nucleoside phosphorylase (PNP) by activating the prodrug fludarabine effectively. Beneficial therapeutic effects were shown in a pancreatic cancer model. Moreover, in the treatment of gemcitabine-resistant pancreatic adenocarcinoma cells, no cross-resistance to both MV oncolysis and activated prodrug was detected [83].

There are several poxviruses that have been studied in pancreatic cancer treatment including vaccinia virus (VACA), myxoma virus (MYXV) and raccoonpox (RCNY). The most widely studied is VACA. The expression of the endostatin-angiostatin fusion protein was confirmed in pancreatic cancer both in vitro and in vivo, with evidence of inhibition of angiogenesis. A novel VACA expressing endostatin-angiostatin fusion protein showed significant antitumor potency in vivo against pancreatic [84]. Survivin is overexpressed by 70–80% of pancreatic cancers, and is associated with resistance to chemotherapy and a poor prognosis. A modified vaccinia Ankara (MVA) expressing murine survivin was recently developed. Vaccination with MVA-survivin in combination with gemcitabine resulted in significant tumor regression and prolonged survival in pancreatic cancer mice. The combination therapy represents an attractive strategy to overcome tumor-induced peripheral immune tolerance, and this effect has potential for clinical benefit in pancreatic cancer [85]. GLV-1h68, a replication-competent virus with mutations in F14.5L, J2R (encoding thymidine kinase), and A56R (encoding hemagglutinin) loci, was reported to be able to infect, replicate in, and lyse pancreatic cancer cells in vitro. It also showed outstanding therapeutic effects and a safety profile in mice with great promise for future clinical development. The combination therapy of GLV-1h68 together with cisplatin or gemcitabine to treat PANC-1 tumors resulted in enhanced and accelerated therapeutic results compared with the virus treatment alone [86]. Hypoxia was reported to contribute to the aggressive and treatment-resistant phenotype of pancreatic cancer. In hypoxic condition viral protein production was not affected. Interestingly, there was an increase in viral cytotoxicity for CFPac1 and MiaPaca2 cell lines after exposure to hypoxic condition. It suggests that the VACA might be a promising agent for targeting pancreatic cancer and potentially other hypoxic tumor type [87]. To enhance the antitumor potency, immunoadjuvant IFN-α was combined with poxvirus vaccine targeting CEA for pancreatic cancer. IFN-α slowed tumor growth, induced CTL activity, and increased CD8+ tumor-infiltrating lymphocytes [88]. In a Phase clinical trial, A VACA expressing tumor antigens CEA and mucin-1 (MUC-1) with three co-stimulatory molecules B7.1, ICAM-1 and LFA-3 (TRICOM) (PAN-VAC-V) was applied in advanced pancreatic cancer. A significant increase in overall survival was detected in treated patients with mild injection-site reactions as the most common adverse events. These data suggest that poxvirus-based vaccine therapy for patients with advanced pancreatic cancer is safe, well tolerated, and capable of generating antigen-specific immune responses [89].

Myxoma virus (MYXV) is a novel oncolytic virus that has been shown to replicate in pancreatic cancer cells. The combination therapy of MYXV with gemcitabine showed robust MYXV replication in a broad range of pancreatic cancer cells and increased oncolysis. In immunocompetent intraperitoneal dissemination pancreatic cancer models, combination therapy resulted in 100% long-term survivors. Therefore, MYXV is an effective oncolytic virus for pancreatic cancer and can be combined with gemcitabine to enhance survival [90]. vMyxgfp, a newly genetically engineered replication-competent oncolytic MYXV, could productively infect, replicate in, and lyse pancreatic cancer cells including the most resistant cell line Capan-2 in vitro, which encourages further investigation of this virus for treatment of the fatal pancreatic cancer [91].

3. Conclusion

The prognosis of pancreatic cancer remains poor with current treatment strategies including surgery, chemotherapy and radiotherapy. A number of viruses have proven to be valuable modalities for pancreatic cancer therapy nowadays. They have been shown to be both safe and effective through different routes of delivery including intratumoral, intraperitoneal and systemic administration in animal models. The therapeutic potency has been improved by manipulation of the viral genome and insertion of foreign transgenes such as cytokines, suicide genes or shRNAs, for therapeutic purpose. Pancreatic cancer associated genes were also introduced into the viruses to enhance the therapeutic specificity for pancreatic cancer and to reduce the potential toxicity as well. Several oncolytic viruses showed promising results in the clinical trials for pancreatic cancer therapy. Synergistic effects of the virus therapy have been demonstrated in combination with current conventional approaches of therapy including chemotherapy and radiotherapy. The virus therapy to date holds great promise to treat pancreatic cancer; however, more efforts are necessary to maximize the benefits for clinical application before the strategy becomes a standard therapeutic agent for pancreatic cancer.

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