

Gene Therapy of Pancreatic Cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumors with a 5-year survival rate of less than 5%. The poor prognosis of the disease is associated with late diagnosis and a high degree of drug resistance has not been overcome during the past decades. Gemcitabine-based regimens are the first line therapy for advanced pancreatic cancer but are not curative. Recent new combination chemotherapies achieved significant benefits but toxicity makes their use controversial. Novel approaches are currently being developed; in particular cancer gene therapies are undergoing preclinical and clinical validation and are the topic of the present review. We will present different ways to design gene therapy against pancreatic cancers that have been validated in preclinical studies. We also reviewed the clinical trials already published or still ongoing.

Keywords: Pancreas; Adenocarcinoma; Gene therapy; Suicide gene; Targeted therapy

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive cancer with a high mortality rate in a time window very near the discovery of the disease. Due to the lack of specific symptoms, the diagnosis is delayed and PDAC is usually detected at an advanced stage of the disease. The prognosis of patients with pancreatic adenocarcinoma is very poor and not improved by the usual chemotherapies, even after surgical resection of the tumor [1]. The 5-year survival is <5%. Surgery offers the only long term survival, but only to a limited number of patients (10 to 20%). Unfortunately, even when resection is possible, a large number of patients show recurrence of the disease suggesting the presence of undetectable micrometastases at the time of surgery [2]. Gemcitabine is the standard first-line chemotherapy since it improves the disease-related symptoms and doubles the survival rate for 18% of the patients [1]. More recently, folfirinox regimen (Irinotecan + Oxaliplatin + 5-Fluorouracil) improved the overall survival of patients with pancreatic cancer, but the side toxicity is still debated to conclude whether or not it is preferable to gemcitabine alone [3]. A recently reported clinical trial shows that it does improve quality of life [4]. Thus, it appears that current therapeutic options for pancreatic cancer are very limited and at best, only improve the palliative treatment of the disease.

The success of targeted therapies in other cancers supported extensive efforts made to identify adjuvant or neoadjuvant therapies capable of improving the prognosis of PDAC, based on the molecular targets involved in cancer progression. Unfortunately, phase III studies have shown limited or even no improvement in patient survival in combination with gemcitabine. For example, 90% of pancreatic adenocarcinomas are mutated in *KRAS*, locked into its active form, bound to GTP. Tipifarnib, an inhibitor of farnesyl transferases, used with gemcitabine, however, failed to improve patients overall survival in phase III trials [5]. Thus far, only erlotinib, an inhibitor of the Epidermal Growth Factor (EGF) receptor 1 has shown moderate improvement in patient survival in combination with gemcitabine. However, in phase III clinical trials, the overall survival was raised of only 0.33 month (about 10 days, [6]). The Vascular Epithelial Growth Factor (VEGF) was targeted with its monoclonal antibody bevacizumab but modest increase of median survival was achieved and phase III trials were terminated early because of lack of benefits of the treatment [7]. Other

targets are under current phase II trials such as inhibitors of hedgehog pathway, inhibitors of SRC or mTOR, associated with new means of drug delivery such as endoscopy ([8] for review). The prognosis is worsened by the lack of benefits of neoadjuvant or adjuvant therapies involving radiations [1].

Because other therapeutic options failed to be efficient in controlling the progression of pancreatic cancers, the field of cancer gene therapy is currently in an active state of preclinical and clinical investigations for this disease. Gene therapy needs to consider concomitantly aspects of safety, specificity and efficiency. The therapy should be specific of the tumor cells, sparing the normal cells. Moreover, the system should reach high numbers of tumor cells to hopefully induce sufficient toxicity to stop the progression of the tumor and hopefully induce regression. To meet these goals, efforts have been focused on choosing the proper therapeutic gene and the safest delivery system.

Correcting altered genes

One approach is to design gene therapy of pancreatic adenocarcinoma based on genetic alterations found in the disease (Figures 1a and 1b). This implies characterization of each tumor at the molecular level before therapy is chosen if most common genetic alterations are not targeted. It was tried to restore the lost expression of a tumor suppressor or to inhibit oncogene expression in pancreatic tumor cells with special attention to the genes that show the highest frequencies of mutation in pancreatic adenocarcinoma [9]. Adenoviral-driven expression of p16-INK4a and p53 has proven efficient in preclinical studies [10]. However, providing p16-INK4 alone was not sufficient [11]. Moreover, as p53 acts as a tetramer, the presence of a mutant inactive form leads to a dominant negative effect limiting p53

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gene restoration efficiency [12]. Restoring a wild type *SMAD4* did not always block tumor cells [13] rendering the use of this tumor suppressor controversial [14]. Many oncogenes can be deregulated and/or mutated in pancreatic cancers. Among them, mutations in the *KRAS* oncogene occur in almost all pancreatic adenocarcinomas [9]. Thus, it is tempting to use direct anti-*KRAS* strategies to inhibit tumor growth (Figure 1b), since disappointing results have been obtained with inhibition of farnesyl transferases [1]. RNAi directed against mutated *KRAS* showed anti-tumor activity [15], limited the aggressive phenotype of the tumor cells [16] and potentiated gemcitabine antitumor activity [17]. Preclinical studies showed encouraging results with viral delivery systems such as oncolytic adenovirus [18]. However, this strategy has not been further tested in phase I clinical trials.

In fact, pancreatic adenocarcinomas present very complex genetic alterations profiles. The Pancreatic Cancer Genome project has analyzed 23,219 transcripts and identified an average of 63 somatic mutations per PDAC affecting 12 core signaling pathways and the overexpression of 500 different genes in 24 tumors [19]. These highly versatile and unpredictable molecular patterns can explain the failure of single gene/pathway targeted adjuvant therapies. It is now critical to test therapies targeting several pathways or therapies that induce specific tumor cell death.

Delivering a Suicide Gene

With the suicide gene approach, a gene that encodes a protein triggering tumor cell death is delivered to the tumor cells (Figure 1c). The expression of the therapeutic gene can directly kill the cells (diphtheria toxin, [20]), or can render the cells sensitive to certain otherwise non toxic prodrugs (Gene Directed Enzyme Prodrug Therapy, GDEPT, [21]), or based on gemcitabine association with the

deoxycytidine kinase:uridine monophosphate kinase fusion gene [22]. The systemic administration of conventional chemotherapies fails to reach sufficiently the pancreatic tumor cells and affects the normal cells. The delivery of therapeutic genes directly in the tumors limits toxicity to normal cells while targeting more toxic drug to the tumor cells. Moreover, some of the GDEPTs produce locally, in the tumor, toxic compounds that can affect neighboring tumor cells which did not necessarily receive the therapeutic gene. It is called the bystander effect.

The ganciclovir (GCV, 2-amino-9[1,3-dihydroxypropan-2-ylloxymethyl]-3H-purin-6-one) is an analog to the 2'-deoxy-guanosine [23]. The thymidine kinase (TK) from Herpes Simplex Virus 1 (HSV-1) shows high activity to monophosphorylate GCV, which is in turn phosphorylated into triphosphate GCV by cellular kinases. The GCV triphosphate is incorporated in place of dGTP during DNA replication leading to DNA polymerase inhibition, double strand breaks and apoptosis [24]. Bystander effect is observed and is dependent on connections through gap junctions between tumor cells. Preclinical studies have demonstrated efficiency of GCV/TK when delivered through various ways such as adenovirus [25], liposomes [26], adenovirus and retrovirus [27,28], oncolytic adenovirus [29]. Recently, we published a report showing the delivery of the HSV-TK gene with a lentivirus pseudotyped to target tumor-specific cell surface antigens [30]. In particular, we showed that the MUCIN-4 antigen was a potent molecule to target anti-cancer therapies for PDAC.

5-Fluorouracile (5-FU) is a chemotherapeutic agent used against pancreatic adenocarcinoma despite severe side effects such as myelosuppression, dermatoses, diarrheas, or cardiac complications. To limit these collateral damages, it is possible to use gene therapy transferring the prokaryotic or yeast cytosine deaminase (CD) able to convert the non toxic 5-Fluorocytosine (5-FC) into toxic 5-FU [21]. Cellular

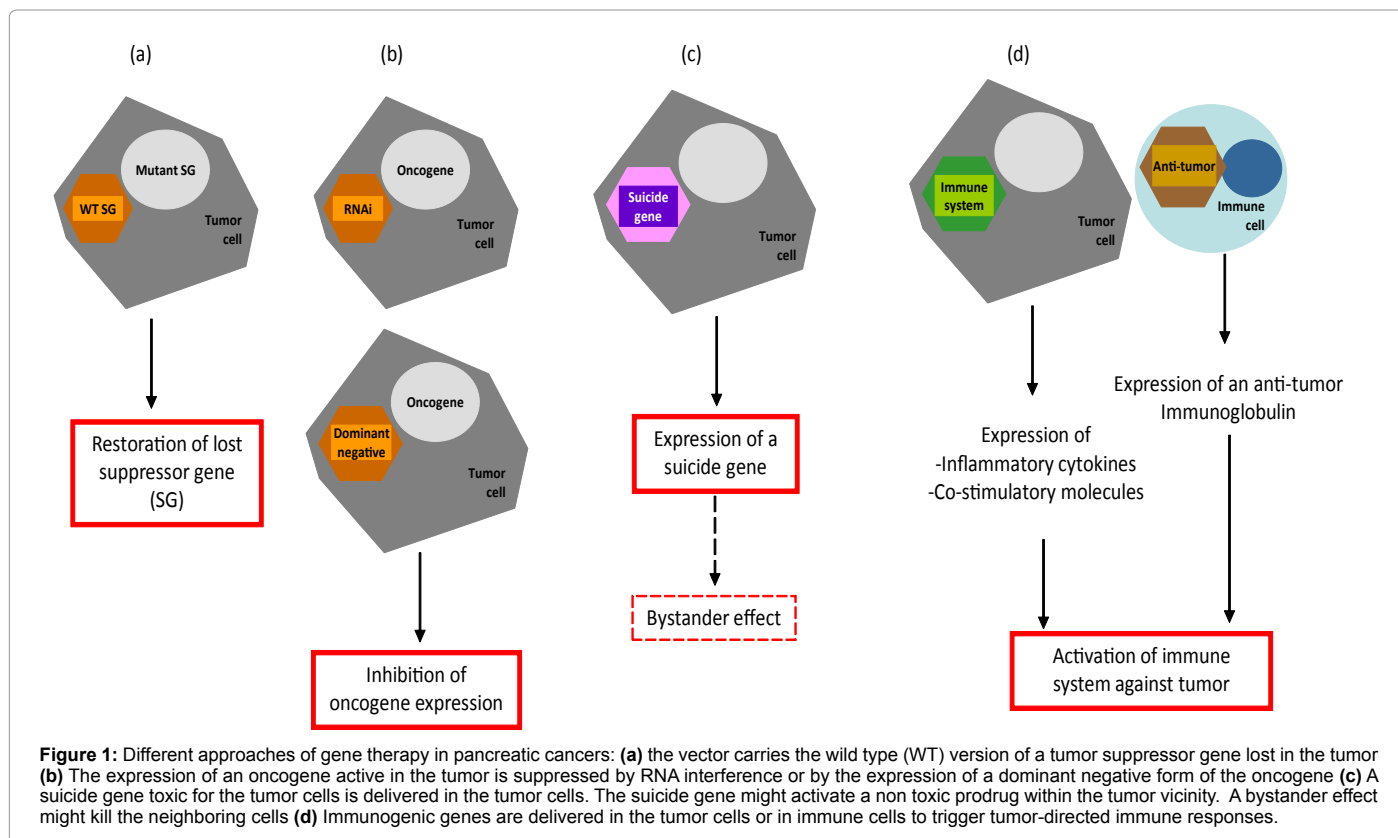


Figure 1: Different approaches of gene therapy in pancreatic cancers: (a) the vector carries the wild type (WT) version of a tumor suppressor gene lost in the tumor (b) The expression of an oncogene active in the tumor is suppressed by RNA interference or by the expression of a dominant negative form of the oncogene (c) A suicide gene toxic for the tumor cells is delivered in the tumor cells. The suicide gene might activate a non toxic prodrug within the tumor vicinity. A bystander effect might kill the neighboring cells (d) Immunogenic genes are delivered in the tumor cells or in immune cells to trigger tumor-directed immune responses.

enzymes convert the 5-FU into 5-Fluorouridine-5'-triphosphate (5-FUTP). 5-FUTP inhibits nuclear to cytoplasm shuttling of RNAs. More importantly, 5-FUTP inactivates irreversibly the thymidylate synthase, limiting the cell supply in dTTP, which forces the 5-FUTP to be incorporated into the DNA where it causes damages to the genome followed by apoptosis [31]. 5-FU also shows bystander effect with simple diffusion of the compound in the vicinity of the tumor, which does not need gap junctions between the cells as for the GCV/TK system [32]. Multiple transfections of CD into the BxPC-3 pancreatic cell line grown into tumors in immune-deficient mice followed by 5-FC administration inhibited tumor growth [33]. Adenoviral CD transfer combined with Death Receptor 5 (DR5) antibody treatment produced additive cytotoxic effect in xenografts models [34]. The development of a mutant bacterial CD carried by an adenovirus gave encouraging results when combined to radiotherapy [35] as already demonstrated under hypoxic conditions [36]. Finally, a recent publication assessed the sensitivity of several pancreatic cell lines to 5-FC/CD *in vitro* and showed that the cells expressing CD were killed by clinical-relevant doses of 5-FC [37]. However, *in vitro* transfer of CD and FURI, encoding uracil phosphoribosyl transferase (UPRT), in several pancreatic cancer cell lines resulted in various sensitivity to prodrug treatment, suggesting that this therapy has little chance to work *in vivo* [38]. This is in agreement with previous clinical data obtained with 5-FU that was found a less potent therapeutic option than gemcitabine [1]. It might be the reason why we did not find any clinical trials completed or ongoing testing the couple 5-FC/CD GDEPT.

Cyclophosphamide (CPA) is a prodrug widely used to treat cancers. It is turned in 4-hydroxyphosphamide by enzymes belonging to the cytochrome P450 family in the liver where it becomes the acroleine and a cytotoxic phosphoramidate binding to DNA and triggering apoptosis [21]. It carries a strong bystander effect. Among the cytochrome P450 family members, the *CYP2B1* gene encodes the most potent enzyme for the transformation of CPA. It was tested in a preclinical study of cell therapy [39]. The 1-year survival was 36% among the 14 treated patients. None of them showed tumor progression and 4 had tumor regression (Table 1). Although published 10 years ago, this approach was not further tested in phase III clinical trials. In the meantime

CPA/CYP2B1 therapy was assessed by gene therapy in preclinical models. CYP2B1 gene transferred with adenoviruses modified to target fibroblast growth factor receptors (FGFRs) using an FGF2-Fab' conjugate was active *in vivo* in human xenografts [40] or more recently *in vitro* with a retroviral system [37].

Besides the exogenous suicide genes suitable for pancreatic cancer gene therapy, it is possible to restore the activity of endogenous apoptotic cascades often interrupted during cancer progression, leading to apoptosis resistance of the tumor cells. In this way, overexpression of pro-apoptotic genes like *BAX* and the tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*) sensitized pancreatic tumor cells to apoptosis and to gemcitabine [41]. As apoptosis is regulated by the balance of activities of several anti-apoptotic and pro-apoptotic factors, the inhibition of inhibitors of apoptosis proteins (IAPs) by antisense approaches sensitized pancreatic tumor cells to *TRAIL*-mediated apoptosis [42] or to chemotherapy [43]. More recently, silencing of XIAP by transfection with XIAP shRNA inhibited the growth of pancreatic cancer cells *in vitro* and *in vivo* [44] and increased chemosensitivity to 5-FU and gemcitabine [45]. Adenovirus-mediated transfer of p53 upregulated modulator of apoptosis (*PUMA*) in pancreatic cells harboring *KRAS* mutations led to tumor growth inhibition [46]. Combined therapy with adenoviral transfer of TNF-alpha, gemcitabine and inhibition of nuclear factor-kappa B (NF-kB) had pronounced antitumor effects [47]. These recent approaches are quite preliminary and deserve further evaluations in the future.

Increasing Immune System Response

One possible option to approach gene therapy of pancreatic cancers is to create an immune response specific to the tumor cells also known as targeted immunotherapy (Figure 1d). This approach can be related to a sort of anti-tumor vaccination. Pancreatic cancers are accompanied by profound changes in immune surveillance programs probably because oncogenic *KRAS*-induced GM-CSF production promotes the development of pancreatic neoplasia by suppressing T cell antitumor response [48]. To regain immunity around the tumor cells, several molecules have been tested for pancreatic cancers such as IL-1, IL-2, TNF-α and IFNs (reviewed in [49]). The aim

Clinical phase	Target cells	Vector	Gene	Outcome	ClinicalTrials.gov identifie	Reference
I/II	Tumor cells	Encapsulated cells overexpressing the therapeutic gene	Cytochrome P450 2B1	14 treated patients: 4 with tumor regression, 10 with stable disease. 1 year survival double than with gemcitabine	Not available	[39]
I	Tumor cells	Retrovirus	Cytocidal N-terminal truncated cyclin G1 (Rexin G)	Treatment well tolerated but no evidence of anti-tumor activity	Not available	[59]
I/II	Tumor cells	Retrovirus	Cytocidal N-terminal truncated cyclin G1 (Rexin G)	Treatment is safe. Dose response relationship between treatment and overall survival	NCT00504998	[60]
I	Tumor cells	Adenovirus	Thymidine kinase	Treatment is safe and needs further validation in phase II trials	Not available	[61]
I/II	Tumor cells	Plasmid DNA	Diphtheria Toxin A chain	Treatment is safe but effectiveness on overall survival needs to be proven	NCT00711997	[64]
I	Patient's T cells	Non replicative canarypoxvirus	CEA & B7.1	Treatment is not toxic. 3 patients out of 12 with stable disease and signs of anti-tumor immune response.	Not available	[65]
I	Patient T cells	Vaccinia virus	CEA & MUCIN-1& co-stimulatory genes	Little adverse effects of the treatment. 5 out of 8 patients had T cell-specific response and increased overall survival	Not available	[66]
I	Tumor cells	Adenovirus	IL-12	Treatment is safe. 5 assessable patients with pancreatic cancer; 2 stable disease, 3 progressive disease	Not available	[67]
III	Tumor cells	Adenovirus	TNF-α	Treatment is safe but not effective	NCT00868114	[68]

Table 1: Completed Clinical Trials of Gene Therapy for Pancreatic Cancer.

Clinical phase	Target cells	Vector	Gene	ClinicalTrials.gov identifie
I	Patient's T cells	Not specified	Anti-CEA Ig	NCT00004178
II	Patient's T cells	Not specified	Anti-mesothelin	NCT01583686
Not specified	Tumor cells	HSV	GM-CSF	NCT00402025
I	Tumor cells	Plasmid DNA + PEI	SST2R+ DCK + UMK	NCT01274455
I/II	Tumor cells	Adenovirus	Thymidine kinase	NCT00638612

SST2R: Somatostatin receptor subtype 2; DCK: deoxycytidine kinase; UMK: uridylylmonophosphate kinase; PEI: polyethylenimine

Table 2: Ongoing Clinical Trials of Gene Therapy for Pancreatic Cancer.

of antitumor immunotherapy is to induce an efficient cytotoxic T lymphocyte (CTL) response against pancreatic cancer cells. IL-2 and B7.1 co-transferred into pancreatic tumor cells led to complete remission of xenografted tumors and to memory immune response [50,51]. *IFNG* transfer with an adenovirus was also efficient in pre-clinical studies [52] and it appeared that the combination of peptide vaccine and gemcitabine provided an additive and synergistic effect, leading to an enhancement of antitumor activity [53,54]. Other IFNs have been tested in immunotherapy approaches. The delivery of *INFA* in carcinoembryonic antigen (CEA)-expressing pancreatic tumor cells resulted in major cytotoxicity through CD8⁺ and natural killer (NK) cells at the tumor site [55]. Moreover, transfer of *INFB* with a lentivirus in xenograft models resulted in tumor progression inhibition [56]. CEA transfer in T cells was tested in a preclinical model to target antitumor response [57].

Clinical Trials Completed and Ongoing

Clinical trials designed to test gene therapy for pancreatic cancer are quite scarce. We interrogated the US National Institute of Health clinical trial and Pubmed databases and selected a few trials completed and also some that are still ongoing that were considered as true gene therapy because they involve the transfer of a therapeutic gene (Tables 1 and 2). There was no trial involving the use of exogenous genes correcting altered genes, probably because pancreatic cancer is the result of numerous abnormalities affecting many genes and therefore, it is unlikely to obtain significant results when focusing on one gene or even one signaling pathway [19]. It appears that most clinical trials have been designed to transfer suicide genes or to direct immune system against the tumor cells (Tables 1 and 2). There is one clinical trial still recruiting, combining the transfer of the deoxycytidine kinase and the uridylyl monophosphate kinase sensitizing the tumor cells to gemcitabine with the transfer of the gene encoding the somatostatin receptor subtype 2 (*SSTR2*), which behaves as a tumor suppressor (Table 2) [58].

Over the trials that have been completed and published, it appears that good clinical efficiency in pancreatic cancer still needs to be demonstrated. In particular, the retroviral transfer of the cytosolic N-terminal truncated cyclin G1 (Rexin-G) did not give any evidence of efficacy in one trial [59] but seemed to have a dose-dependent response in another trial (Table 1) [60].

Despite the numerous preclinical studies that have tested GCV/TK, we found only three clinical trials testing this GDEPT with adenoviral transfer (Table 1). However, among all the possible gene therapies, GCV/TK is the most frequently tested for pancreatic cancers. One of the trials is still recruiting and the results are not available. Two other trials reported during the American Society of Clinical Oncology (ASCO) conferences in 2011 displayed encouraging results that will probably lead to phase II studies (Table 1) [60]. Noticeably however, preclinical studies showed that GCV/TK treatment can lead to resistance, probably because some of transferred genes can be lost a

while after the beginning of the treatment [61,62]. Moreover, activation of the checkpoint kinase 1 (chk1) cell cycle regulator might control cell sensitivity to GCV/TK cytotoxicity [63]. These aspects of the GCV/TK approach might limit translation to phase III trials. The CPA/CYP2B1 GDEPT did give encouraging results in a trial involving 14 patients [39] but still needs confirmation. The same statement can be done for the use of the diphtheria toxin A chain published recently [64].

Increasing immune system approaches were not more successful in delivering evidence of efficacy. The viral transfer of CEA and B7.1 into T cells was tested in a phase I trial but the results were not spectacular with only 3 out of 12 stable disease [65]. More promising results have been reported when several tumor-directing antigens, including CEA, together with co-stimulatory molecules were delivered to T cells with poxviruses since the overall survival was significantly increased in the patient demonstrating efficient immunization [66]. Providing the tumor environment with cytokines such as IL-12, or TNF- α did not result in regression of the tumors [67,68]. It will be interesting to see whether other genetic manipulation of patient's T cells or the transfer of GM-CSF in the tumors will be more efficient when the clinical trials involving these approaches will be completed (Table 2).

Conclusion

Despite the numerous pre-clinical studies testing pancreatic cancer gene therapy, very few strategies have been transferred in clinical trials. Among the trials that have been published, only a few displayed encouraging results. This demonstrates the challenges of translational medicine, and the poor predictability of preclinical science when tested in the human diseases. In addition, this overall failure to provide efficient therapy highlights the need of using multiple agents simultaneously to elicit tumor cell killing. However, as very few trials have been published, it is still reasonable to think that gene therapy remains a promising strategy for pancreatic cancer because most of the "conventional" anti-cancer weapons have failed to cure or to improve survival. Combining different gene therapy approaches (transfer of several therapeutic genes) with current modalities may be the way to go in the future design of new clinical trials. Moreover, we would like to note that anticancer virotherapy can also be a promising tool in therapies against pancreatic cancers. It is designed to use oncolytic virus able to replicate only in the tumor cells and not in the normal cells. It combines gene therapy to increase anti-tumor toxicity. The oncolytic viruses are armed i.e. they express an exogenous gene increasing toxicity (suicide gene) or anti-tumor immune response. This very interesting and very promising approach has been recently reviewed [69].

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