

Review Article

Gene Therapy for Treatment of Pancreatic Cancer

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***Corresponding author:** Mishra B, Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi - 221005, UP, India**Received:** August 05, 2014; **Accepted:** September 16, 2014; **Published:** September 16, 2014**Abstract**

Pancreatic cancer is one of the most dreadful disease having high morbidity and mortality rate with limited success in treatment option. Even after chemotherapy, radiotherapy and surgical interventions, long-term survival remains a remote possibility. The identification of appropriate targets for exploiting the novel modalities, an alternative to existing adjuvant therapies is the need of hour. The advancement in identification of genetic and molecular target controlling the critical pathways in pancreatic cancer provides deep insight for the development of newer strategies. Gene therapy approaches are currently being explored for the treatment and prevention of pancreatic cancer deaths by engineered novel delivery systems. In the present review, an overview of principles behind use of gene therapy including molecular targets, various delivery vectors and gene therapy approaches in context to pancreatic cancer, including *in-vitro*, *in-vivo* and clinical studies are discussed.

Keywords: Pancreatic cancer; Gene; Therapy; Delivery vector; Chemotherapy

Abbreviations

DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; GDP: Guanosine Diphosphate; GTP: Guanosine Triphosphate; INK4: Inhibitor of cyclin-dependent kinase 4; CDK: Cyclin-Dependent Kinase; TGF- β : Tumor Growth Factor- β ; EGFR: Epidermal Growth Factor Receptor; FGF: Fibroblast Growth Factors; CAR: Coxsackie-Adenovirus Receptor; AAVs: Adeno-Associated Viruses; PLL: Poly-L-Lysine; PEI: Polyethyleneimine;

MSCs: Mesenchymal Stem Cells; TSPs: Tumor-Specific Promoters; hTRET: Human Telomerase Reverse Transcriptase; GDEPT: Gene-Directed Enzyme Prodrug Activation Therapy; HSVtk: Herpes Simplex Virus Thymidine Kinase; IL: Interleukins; APCs: Antigen Presenting Cells; TIMP: Tissue Inhibition of Matrix Metalloproteinases; TRAIL: Tumour Necrosis Factor-Related Apoptosis-Inducing Ligand

Introduction

Pancreatic cancer is one of the most ravaging diseases prevalent in today's time, ranked as fourth common cause of cancer deaths and tenth in new cancer cases. The treatment and management of this disease by using existing conventional therapies has faced difficulties in providing complete cure [1]. Despite advancements in the diagnostic techniques, early stage cancer prognosis is still one of the most challenging and grave problems; with a post-diagnosis 5 year survival rate of only 4%. Hence, introduction of new modalities in the treatment option is necessary [2]. The identified precursor for pancreatic cancer is the multiple genetic mutations which result in disinherited growth, evasion of host immune response, sustained angiogenesis and avoidance of apoptosis and metastasis that can effectively be targeted for the therapeutic interventions [3]. Chemotherapeutic treatment option has been proven to be effective in palliative treatment only. However, overall outcome is poor due to development of resistance with median survival of less than 3 to 5 months [4,5]. Most of the patients suffer from locally advanced

or metastatic cancer. Among them, limited numbers of patients are suitable for surgical resection which provides the opportunity for a long-term disease-free state but not promised. These shortcomings of existing treatment options indicate the need of novel therapies.

Recent progress in the molecular research provides an insight in pancreatic cancer associated genes with their expression profiles and mutation in cancer cells as well as genetic targets for development of novel therapeutic strategies, either alone or in combination with existing conventional cytotoxic chemotherapies. These allow improvement in treatment outcomes and also reduce toxicity as well as problem of cross-resistance, which generally happens with standard radiotherapy and chemotherapy.

Gene therapy treatment approach is based on the delivery of genetic material i.e., exogenous nucleic acid into cancer cells of a patient, to eradicate the cause of cancer by manipulating intracellular genetic material. The gene linked with regulatory DNA sequences is carried by vectors, either viral or non-viral to transport into the target cells where it expresses. The transgene expression might occur in every transfected cell or selectively targeted cells, where specific activated transcription factors are present, which interact with tissue selective or tumor selective promoter/enhancer elements [4,6]. The theoretical basis for gene therapy is the assumption that expression, restoration, elimination or inhibition of the activity of a particular gene of interest will reverse the malignant phenotype and hence, the growth of cancer cells will be prevented or inhibited. The effectiveness of gene therapy involves the technical ability to inhibit or restore gene products in most of the tumour cells [7,8]. The key elements of the efficient gene therapy are shown in Figure 1.

Various strategies have been utilized for gene therapy including inhibition of activated oncogenes by antisense strategies, restoration of the functioning of tumour suppressor genes, gene-directed prodrug activation therapy and the use of replication-selective oncolytic viruses, etc. Gene transfer can possible by number of means, including use of viral vector and non-viral vectors. Most of the developments in

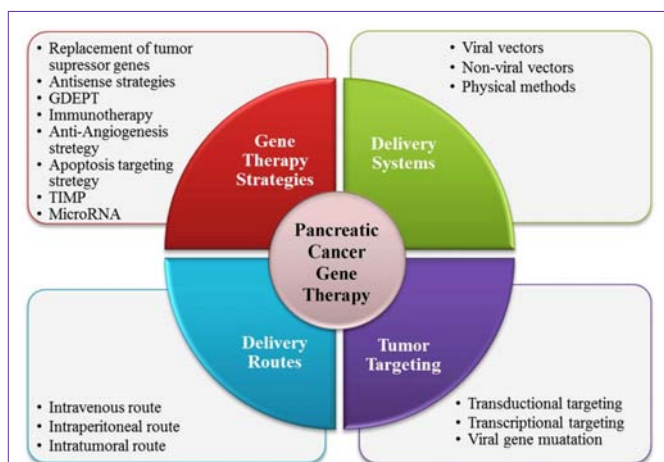


Figure 1: Key elements in the development of efficient pancreatic cancer gene therapy.

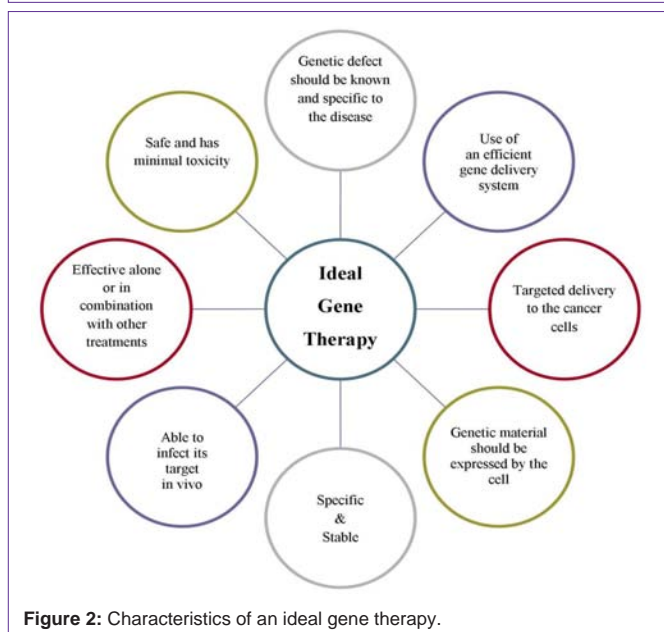


Figure 2: Characteristics of an ideal gene therapy.

pancreatic cancer gene therapy are in the pre-clinical phase of studies and few have progressed to early phase clinical trials [4]. However, for increasing the clinical application of the gene therapy, it should have ideal characteristics Figure 2.

Molecular Targets of Pancreatic Cancer

Intensive research has been done over the past two decades for identification and characterization of molecular alterations that generally occur in pancreatic cancer. The pancreatic cancer is one of the most complex malignant cancer, it occurs as a result of accumulated genetic alterations with more number of mutations as compared to other type cancers. It involves the mutation of five or more genes in its genetics. Mutations can be possible in oncogenes, tumour-suppressor genes, or maintenance genes (i.e., growth factors) which may subsequently activate oncogenes or inactivate tumor suppressor genes and lead to malignant cancer (Table 1).

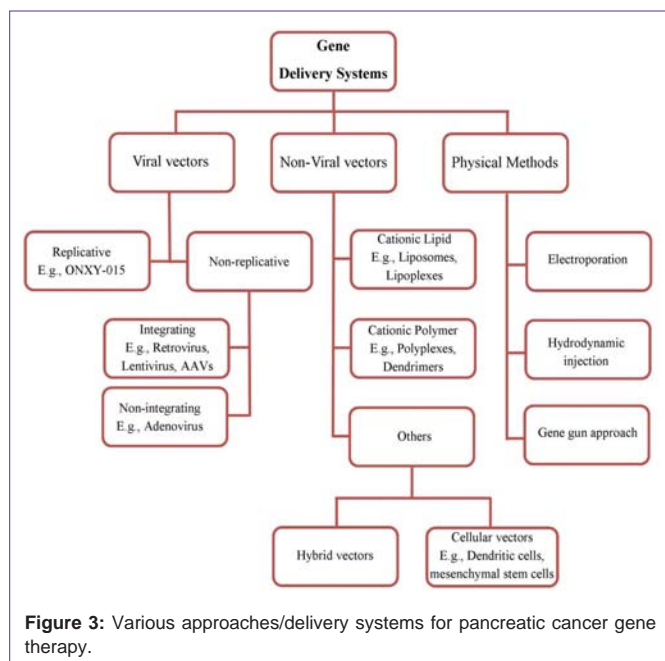
Oncogenes

Oncogenes are the number of the genes that exhibit increased

Table 1: Genetic and molecular targets for pancreatic carcinoma [9,10].

Gene	Frequency of mutation/expression (%)
Oncogenes	
Mesothelin	90-100
KRAS	95
CCK-B receptor	95
MUC1	90
Bcl-xL	90
CEA	85-90
LSM1	87
BIRC5 (survivin)	77-94
EGFR	69
Bcl-2	23
AKT2	10-20
MYB	10
Tumor suppressor gene	
CDKN2A	90
ARPC5	>90
p16 ^{INK4A} (MTS1)	85
p53	50-75
SMAD4	55
BRC A2	7-10
pRb	6
LKB1/STK11	5
MKK4	4
MAP2K4	4
TGFBR2 (MSI1 positive)	3
MLH1	3
TGFBR2 (MSI1 negative)	1
ALK5	1
TGFBR1	1

biological activity as a consequence of mutation. The RAS gene is the most commonly detected oncogene in human cancer including pancreatic cancer. The main responsible RAS gene for all of the pancreatic cancer mutation is K-RAS which resides on chromosome 12p13 and constitutively mutates up to 95% of adenocarcinomas [11,12]. The mutation results in the activation of effector proteins that differ from those involved in the normal K-RAS signaling system [13]. The gene encodes membrane associated guanine nucleotide binding signal transduction proteins; p21, which regulates various cellular functions including cell growth, proliferation and differentiation [14]. The point mutation in K-RAS gene during the early stage of pancreatic carcinoma results in a mitogenic stimulation of cellular receptor tyrosine kinases, which allows fusion of phosphate to RAS-GDP to form an active RAS-GTP. This in turn promotes increase in signal transduction resulting in eventual gene activation and hence, uncontrolled cell growth and survival [15,16]. The c-erbB-2 and c-myc are the other oncogenes occasionally responsible for pancreatic cancer [17].



Tumor suppressor genes

The inactivation of the tumor suppressor genes results into the loss of control of vital negative regulators of cell proliferation which actuate the uncontrolled cell growth. A number of tumor suppressor genes have been identified which are involved in the pathogenesis of pancreatic cancer including p53, members of the INK4 family and DPC4/SMAD4. The p53 is most commonly mutated tumor suppressor gene in pancreatic cancer residing on chromosome 17p [18,19]. It encodes 53-kD nuclear phosphoprotein which modulates the expression of an array of genes that serves to hold the basic functioning of normal cell including cell cycle regulation, arrest, apoptosis, differentiation, DNA surveillance and repair [20]. Another tumor suppressor gene belonging to the INK4 family of the pro-mitotic complex cyclin-dependent kinase (CDK) inhibitors is p16. It resides on chromosome 9p and is involved in pancreatic cancer. The p16 gene prevents phosphorylation of the retinoblastoma protein via binding with the cyclin-CDK4 complex, thereby arresting the cell cycle at the G1/S phase. The loss of p16 activity prevents the binding with cyclin-CDK4 complex, which results in uncontrolled cell growth [21]. The functionality of p16 gene is depressed in >80% of pancreatic adenocarcinomas which can serve as an important target for genetic correction in pancreatic cancers [12]. The homozygous deletion of chromosome 18q21 resided Locus 4 gene (DPC4/SMAD4) is also responsible for the 30% to 50% of pancreatic cancer. It encodes protein for signal transduction of the tumor growth factor (TGF) [22].

Growth factors

The number of growth factors including, fibroblasts growth factors (FGF), TGF- β and the epidermal growth factor receptor (EGFR) with their ligands have actively been involved in pancreatic cancer. The FGF are essential for normal cell functioning including cell differentiation during tissue repair, mitogenesis and angiogenesis. It consists of 19 homologous polypeptide growth factors. Among all, FGF 1-5 and 7 are found to be over expressed in the pancreatic

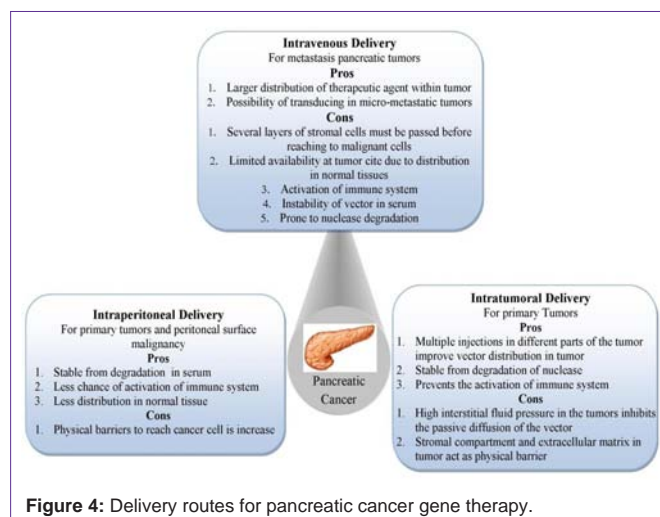
cancer [23,24]. Another growth factor is EGFR, which upon binding, dimerizes and transphosphorylates tyrosine residues, that allows for signal transmission using various cascades. The increase in the levels of EGFR as well as its closely related receptors, such as HER2 and HER3 are responsible for the pathogenesis of human pancreatic cancer [25,26]. Moreover, several ligands related to the EGFR such as heparin binding EGF-like growth factor and TGF- α are also over expressed in pancreatic cancer [27]. The TGF- β also has been found over expressed in pancreatic cancer which upon activation transphosphorylates intra cytoplasmic proteins SMAD2 and 3, which allows to form complex with SMAD4 that serves as transcriptional activator. SMAD6 and 7 are the members of same family that are used to inhibit phosphorylation of the SMAD2 and 3 [28].

Gene Delivery System

Mainly three predominant types of approaches have been utilized to achieve the effective gene delivery. They include viral vectors, non-viral vectors and physical methods (Figure 3) [29]. The most efficient gene transfer till date has been achieved by using viral vectors; hence they are widely used in cancer gene therapy protocol. Further, the viral vectors not only carry genes efficiently into the cells, but also some of the viruses are able to replicate in and destroy tumor cells which are known as oncolytic viruses. However, they suffer from some disadvantages such as potential toxicity, immunogenicity, smaller sized foreign DNA incorporation efficiency and need of packaging cell lines for production, which limits their wide applicability. Whereas non-viral vectors or physical methods are beneficial in terms of manufacturing, handling, no risk of recombination, low immunogenicity and capacity to insert large DNA. However, they also suffer from loopholes such as less effectiveness and lack of targeting potential. Recently, mesenchymal stem cells and hybrid vectors have been studied as a novel gene delivery system. Moreover, the choice of the optimal delivery route also greatly influences the gene therapy outcomes. The various delivery routes with their pros and cones are shown in Figure 4.

Viral vectors

Adenovirus: The adenovirus belongs to the family adenoviridae. It possesses an icosahedral protein shell enveloping approximately 34-48 kb long, linear, double-stranded DNA genome. They are about



70-90 nm in diameter. Among all 51 serotypes, adenovirus type-2 and type-5, belong to subgroup C and are commonly used vectors for gene therapy as well as oncolytic agents. They are genetically stable, conformable to high-titer production as well as purification (10^{13} particles/ml), able to express gene of interest and efficient at entering in both, cell and nucleus. By deleting E1 and E3 genes, the viral replication and immune response stimulation become incompetent. Upon deleting, they are able to carry up to 7.5 kb of foreign DNA. Recently, conditionally replicative adenoviruses have been engineered for cancer therapy by retaining its E1A region which, allows for replication and are able to spread within tumor tissues. This strategy has been successfully used for pancreatic cancer *in-vitro* murine model [30]. Moreover, the alteration has been carried out in adenoviral envelope fibers to increase its attachment in CAR receptor deficient pancreatic ductal cells [31,32].

Retrovirus: Retroviruses are the single stranded RNA containing enveloped viruses, which upon deletion of selected gene become non-replicative and non-immunogenic. It contains the gene such as env (envelope – encodes surface protein which determines specificity of cell type), gag (group-specific antigen – encodes core and structural proteins) and pol (polymerase – encodes integrase, protease and reverse transcriptase), psi (encodes for packaging signal). It creates cloning capacity of 8 kb by deleting gag, pol and env genes [33]. Upon infection to target cell, they release viral content in host cell, where reverse transcriptase transcribes the RNA into DNA, which integrates into host cell's genome and expresses the transgene of interest for long term. The moloney murine leukemia virus has been used as gene delivery vector for pancreatic cancer [34]. Also, the attempt has been made to modify env gene as well as packaging cell line for improving cell specificity to target the vector [35,36].

Lentivirus: Lentiviruses are group of retroviruses which can replicate in non-mitotic cells such as human immunodeficiency virus type-1. The vectors only deliver therapeutic gene to the target cells and are able to integrate in both replicating and non-dividing cells. However, integration of the therapeutic gene into the viral genome occurs randomly. Liu et al. studied the lentivirus mediated RNA interface targeting the high mobility group A1 over expressed pancreatic cancer cell line, for increasing the sensitivity of gemcitabine [37]. Although, no clinical trials using lentiviral vectors have been conducted, but vectors based on lentivirus may prove an attractive alternative for pancreatic cancer gene therapy.

Adeno-associated viruses (AAVs): AAVs are small, non-enveloped, non-immunogenic, non-pathogenic, single stranded DNA containing parvoviruses. They mandatorily require the coinfection with herpes virus or adenovirus for successful replication. The wild type version integrates on to the chromosome 19 of the host genome. The engineered AAVs lose specificity and replication potential by deletion of the cap and rep genes which encodes for structural and viral replication proteins, respectively. The deleted space is able to carry 4.5 kb of foreign gene. They are efficient in transducing both replicating and quiescent cells. Their low cloning potential can be overcome by coinfection with two AAVs, each carrying half gene of interest. Long term transgene expression by using AAVs in transduce tissue has been reported [38]. AAVs based vectors have been successfully used in the treatment of pancreatic cancer [39,40].

Replication-competent oncolytic adenovirus vectors: All the above viral vectors are replication incompetent. Hence, the transduction of target cell does not subsequently transduce in neighboring cell. To achieve the desired goal, replication competent viral vectors have been evolved. They replicate preferentially to increase number of transducing cells, and therefore lyse cancer cells while sparing healthy tissue [41]. The ONYX-015 is a mutant adenovirus which preferentially replicates in tumor cells lacking functional p53. It is made by deleting of E1B gene from the genome of adenovirus. It kills tumor cell by general lytic action of the replicating adenoviruses within tumor cells. In a phase I/II clinical trial, intra tumoral injection of ONYX-015 by using endoscopic ultrasonography in combination with intravenous gemcitabine in patient showed effective results [42]. The use of ONYX-015 has proven safe enough and has successfully entered in phase-III clinical trials [43,44]. These promising results indicate that oncolytic virus therapy could be a practical approach for treatment of pancreatic cancer.

Non-viral vectors

Liposomes: Liposomes are consisting of spherical lipid vesicles with a bilayer membrane structure composing of amphiphilic lipid molecules. Various cationic lipids have been used for the preparation of cationic liposomes. The anionic DNA binds with cationic lipid by electrostatic interactions and forms complex, which is known as lipoplex. They offer protection during the gene delivery. The cationic charge favors the fusion of liposome with the target cells, which enables endocytosis and subsequent delivery of DNA to the cytoplasm, by dissociation of anionic lipids of the cell. However, the problems such as transient transgene expression due to controlled entry into nucleus, lack of targeting specificity and physical instability limits its applications [45]. The targeting problem can be overcome by direct delivery to the organ of interest or by using tumor specific promoter to regulate expression of desired gene. Intra peritoneal administration of lipoplexes also found to be effective in peritoneal dissemination of pancreatic cancer containing mice model [46,47].

Polyplexes and dendrimers: The cationic polymers such as heterogeneous poly-L-lysine (PLL) [48], polyethyleneimine (PEI) [49,50] and oligopeptides [51] get bound with the DNA and form a polymer-DNA complex which is known as the polyplex. Dendrimers are the highly branched synthetic polymers, having spherical shape. They can exist in linear or branched polymer of varying length, but linear polymers are resistant to degradation, more efficient for DNA condensing and exhibit enhanced endosomal uptake [45]. The target specificity of the Polyplexes can be increased by incorporating the receptor-specific antibody or ligands protein. The PEI is the most commonly used high density amine group containing polymer which can easily attract water, causing rupture of the endosome, thereby releasing DNA in cytoplasm. Plasmid complexes with PLL and chimeric protein for targeting the EGF receptor showed elevated expression of plasmid as compared to non-modified Polyplexes [52].

Physical methods for delivery of naked DNA

Naked DNA can easily be developed by the bacteria in the form of plasmid which has unbounded therapeutic insert size. However, lack of tumor specificity and low capability of transgene expression makes it less efficient compared to adenovirus or AVVs. The simplest method for delivering naked DNA is direct local injection to target

sites. DNA are either complexes or coated with the gold particles and are bombarded on the target tissue by accelerating to high speeds using a vacuum pump or a helium propellant, which results in direct penetration through cell membrane and initiation of the effect of foreign DNA. This technique is known as gene gun approach. Various techniques have been developed for improving the uptake of DNA by increasing the cellular permeability. By using the ultrasound and the electroporation techniques, higher cell membrane permeability can be achieved. The naked DNA also can be delivered by hydrodynamic injection which involves the rapid injection of large volumes of genetic material using high pressure. The high pressure on the endothelium helps in improving uptake by transient localized occlusion of blood vessels [53]. However, hydrodynamic gene delivery is well tolerated by rodents only and is not a successful option for humans.

Other methods

Hybrid vectors: Hybrid vectors have been engineered to overcome the difficulties associated with both viral and non-viral vectors. Virosomes are the type of hybrid vectors which comprise of the liposomes and viral antigens (e.g. influenza virus) embedded in the lipid bilayer. They have added advantage of both the vectors such as improvement of cellular binding of particles due to liposomes and the ability to stimulate the host cell anti-tumor immune response due to viral antigens.

Mesenchymal stem cells: Mesenchymal Stem Cells (MSCs) are non-hematopoietic precursor cells derived from bone marrow, which have the function of maintaining and regenerating connective tissue via engraftment. They have drawn significant attention due to their accessibility, tumor-oriented targeting capacity, and the feasibility of auto-transplantation [54]. Its multi potentiality makes it a common choice as vector for efficient gene therapy. The MSCs have successfully been used for gene delivery in glioma, melanoma and breast cancer [55]. Lentivirus-transduce MSCs have been used in targeting human orthotopic pancreatic tumor xenografts in nude mice models [56].

Targeting of Pancreatic Cancer Cells

Effective gene therapy in pancreatic cancer mainly depends on the transduction efficiency of the desired gene and its selectivity for the targeting cells. Various efforts have been made for effective gene therapy by modifying the vectors for targeting the pancreatic cancer cells. The tumor targeted delivery of gene with the help of viruses is achieved by two ways: either surface modification (i.e., transductional targeting) or by utilizing tumor or tissue-selective gene promoters, which helps to express within the viral genome (i.e., transcriptional targeting).

Transductional targeting

The vector tropism is modified by target cell specific moieties which are able to target and infect the tumor cells. Various approaches such as genetic modification, pseudotyping, molecular conjugates etc. have been used previously. Genetic modification involves modification of the viral proteins which participate in the viral entry inside the cell. The viral entry involves the binding with the CAR receptor which is found in limited numbers in pancreatic cancer cells, as a consequence other cellular receptors are targeted [57,58]. The improved transduction in pancreatic cancer was observed by introducing the Arg-Gly-Asp (RGD) peptide into the HI

loop of the fiber protein targeted adenovirus [59,60]. Pseudotyping or incorporation of the chimeric fiber in the genome of vector showed enhanced transduction efficiency [61,62]. High transduction efficiency in human pancreatic carcinoma has been observed by pseudotyping of the enveloping glycoproteins such as vesicular stomatitis virus glycoprotein of retroviral vectors [63]. Adenovirus also has been modified by various molecular conjugates which link the vector with specific cellular receptors by one adenoviral vector recognition part and other receptor identification part [60]. Moreover, transferrin targeting of liposomes conjugated with the single-chain antibody fragment showed enhanced efficiency of gene transfer in pancreatic cancer [64].

Transcriptional targeting

Tumor-specific promoters (TSPs) such as the midkine, cyclooxygenase-2, cancer-specific progression elevated gene-3 promoter, urokinase-like plasminogen activator receptor, human telomerase reverse transcriptase (hTRET) etc. are used for transcriptional targeting in pancreatic cancer cells. TSPs have been used to drive the E1 and/or E4 adenoviral genes, thereby controlling the gene expression and viral replication in pancreatic cancer cells [65-68]. The carcinoembryonic antigen promoter or the tissue specific insulin promoter with modified adenoviral vector has been used to target TK gene expression in pancreatic tumor [69]. Moreover, intravenous administration of liposomes containing the modified cholecystokinin type-A receptor promoter (CCK/Mpd) driven Bik mutant (Bik-DD, T33D/S35D) exhibited pancreatic cancer specific activity in nude mice xenograft model [70].

Gene Therapy Strategies

Antisense strategy

This strategy aims to prevent the transcription, translation, or processing of cancer-associated genes. It involves the production of oligonucleotides which are short sequences of deoxynucleotides and bind to target DNA or RNA sequences in complementary fashion. The binding results in inhibition of gene expression, thereby inhibition of the production of proteins. However, it suffers from some problems such as limited and nonspecific delivery of oligonucleotide as well as stability issues *in-vivo*. Funatomi et al. studied the delivery of antisense oligonucleotide to pancreatic cancer cell line with overexpressed amphiregulin, a ligand of EGFR and found dose dependent inhibition of tumor cells [71]. The ribozymes are the catalytic RNA having endonuclease activity and they can also be used to target specific RNA molecules. They have been shown to be effective modulators of gene expression in pancreatic cancer [72].

Replacement of tumor suppressor genes

The replacement of tumor suppressor genes such as p53, p16 and SMAD4/DPC4 is one of the obvious strategies for gene therapy. The human wild type p53 gene has successfully been transduced in the pancreatic cancer cell line by using adenoviral and retroviral vectors, resulting in growth inhibition and induction of apoptosis [73-76]. In addition, proapoptotic gene p73, upon over expression binds with p53 DNA and activates p53 genes which induce cell cycle arrest and apoptosis [77]. The p16 gene transduction was also observed in the various pancreatic cancer cell lines [78]. The transfer of SMAD4 gene by using adenovirus to pancreatic cancer cell line deficient of SMAD4

showed restoration of expression of SMAD4 with tumor growth inhibition [79].

Gene-directed enzyme prodrug activation therapy (GDEPT)

It is also known as the suicide gene therapy. It involves the delivery of the desired gene to tumor cells which upon expression, activates the enzyme. Subsequently, a non-toxic prodrug is administered which is activated by the enzyme, produced in the tumor cells only. The selective accumulation of the activated drug in higher concentrations in tumor cells produces apoptosis in tumor tissues without affecting other normal tissues. The herpes simplex virus thymidine kinase (HSVtk)/ganciclovir system is the most well-known paradigm for suicide gene therapy. HSVtk monophosphorylates the guanosine analogue of ganciclovir, which gets converted into triphosphorylated form by cellular guanylate kinases and blocks the DNA synthesis thereby, inducing apoptosis. Adenoviral delivery of HSVtk along with ganciclovir in human pancreatic cancer cells bearing nude mice showed effective results [80]. Wang et al. showed the decrement in survival of tumor cells treated with suicidal gene therapy [81]. Makinen and coworkers similarly observed constructive results *in-vitro* and *in-vivo* [82]. Another kind of GDEPT therapy involves the delivery of cytosine deaminase enzyme gene by linking with oncogene ErbB2 promoter along with 5-fluorocytosine [83].

Immunotherapy

The immune system plays an important role in control of the growth of cancer cells. However, pancreatic cancer cells are relatively poor immune stimulators thus are unable to activate the host immune system. Various attempts have been made by using recombinant DNA technology to increase the level of immunogenicity of tumor cells to activate the immune system against the cancer cells growth. Immunotherapy may be passive or active. Passive immunotherapy includes the use of *in-vitro* developed antibodies or effector cells as anti-tumor agent whereas active immunotherapy includes the use of vaccination to activate anti-tumor activity. Peplinski et al. delivered recombinant vaccinia virus in mice having pancreatic cancer which resulted in encoding of human interleukin-1 (IL-1) and decrement in tumor size [84]. Various strategies have been used for immunotherapy such as genetic modification of the cancer cells in such a way that they express cytokines or costimulatory surface molecules which attract antigen to tumor site and activate killer T cells. The murine pancreatic cancer cells have been transduced retrovirally to express IL-2, IL-4, IL-6, IL-12, IL-15 and TNF- α which could induce an antitumor immune response, resulting in tumor arrest and long-lasting immunity [85-88]. The enhanced cytotoxic T lymphocyte response, thereby, enhanced immune response was observed by injecting the antigen presenting cells (APCs) with synthetic mutant RAS peptides in pancreatic cancer patients [89,90]. Immunotherapy using vaccine has not been much explored. However, delivery of cancer associated antigens with recombinant viral vectors as well as other immune stimulatory genes to produce an antigen-based vaccine is under development [12,91].

Anti-angiogenesis strategy

Tumor growth is dependent on angiogenesis i.e. new blood vessel formation which involves the VEGF family of proteins and receptors. They are commonly over expressed in 90% of pancreatic

cancers. The inhibition of angiogenesis by replication-competent adenovirus is one of the strategies to suppress the tumor growth. The anti-VEGF ribozyme mediated transfection in human pancreatic cancer cells showed suppression of growth and metastatic potential [90]. As the soluble form of flt-1 VEGF inhibits the activity of VEGF, the adenovirus encoding soluble flt-1 VEGF was delivered in rodent bearing pancreatic cancer and was found to inhibit proliferation of tumor [92]. Natural killer transcript 4 is an antagonist of HGF which binds with the c-Met-encoded receptor, commonly over expresses in 61-87% of pancreatic cancers. By inhibiting the HGF binding, angiogenesis can be arrested in tumor cells [93]. AS-3, VEGF antisense oligonucleotide also has been tested in mice implanted with human pancreatic cancer cells and showed tumor suppression [94].

Tissue inhibition of matrix metalloproteinase (TIMP)

Matrix metalloproteinases are overexpressed in pancreatic cancer and are responsible for degradation of basement membrane, thereby developing local invasion and metastases [95]. The pancreatic cancer cell line has been transduced with a vector coding for the TIMP-1 and was found to inhibit tumor growth with decreased level of implantation, metastasis, and angiogenesis [96].

Apoptosis targeting strategy

Apoptosis also known as programmed cell death, frequently associates with the human malignancy and therefore, is suitable target for cancer treatment including gene therapy. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is well known for producing apoptosis in tumor cell without affecting normal cells. Adeno viral vector driven by hTRET promoter suppresses tumor growth in Mia-Pa-Ca2, BxPc3, Panc1 and AsPc1 pancreatic cancer cell lines [97]. It also produces synergistic apoptosis in pancreatic cancer cell line when given in combination with gemcitabine [59]. The bcl-2, an antiapoptotic gene is highly expressed in most of the pancreatic cancers [98, 99]. Followed by delivering the bcl-2 specific siRNA, transfection, antiproliferation and proapoptotic effects have been seen in pancreatic cancer cells without affecting normal one [100].

Micro RNAs

The modulation of micro RNA function is a potential strategy to kill tumor cells as they control gene expression for the various physiological processes such as proliferation, differentiation and apoptosis. Micro RNAs are ~22 nucleotides containing small, endogenous, non coding RNA molecules, which might act as tumor suppressors or down regulating oncogenes [101]. More than 100 miRNA precursors such as miR-10, miR-21, miR-155, miR-106a, miR-34a and miR-127 are aberrantly expressed in pancreatic cancer [102]. The increased apoptotic responses and sensitivity to gemcitabine were observed by antisense inhibition of miR-21 in pancreatic cancer [103]. However, limited studies have been conducted by using micro RNA treatment approach in pancreatic cancer.

Future Perspective

Gene therapy allows an incredible diversity of treatment possibilities. This diversity can be utilized to complement traditional therapies, by acquiring the radically new frontiers for effective treatment. Current gene therapy trials have demonstrated statistically significant survival improvements in preclinical studies. However,

Table 2: Clinical trials of gene therapy for treatment of pancreatic cancer.

Type of pancreatic cancer	Vector	Therapeutic Agent	Clinical Trial Phase	Outcome
Inoperable pancreatic cancer	genetically modified cells	CYP2B1 gene followed by ifosfamide administration	Phase I/II	Median survival was doubled and one-year survival rate was three-times better.
Locally advanced and metastatic pancreatic cancer	Rexin-G (retroviral vector)	Retroviral vector bearing cytotoxic dominant negative mutant of human cyclin G1	Phase I/II	Safe, well tolerated and dose-response effect between overall survival and Rexin-G dosage, with a 28.6% one year survival in gemcitabine-resistant pancreatic cancer.
Pancreatic cancer	-	Antitumor vaccination using alpha(1,3) galactosyltransferase expressing allogeneic tumor cells engineered by retroviral transduction	Phase I/II	Study completed but results yet not published (clinical trial registration number NCT00255827)
Adenocarcinoma of the pancreatic cancer	-	allogeneic pancreatic tumor cells genetically modified to express the GM-CSF factor	Phase II	Study completed but results yet not published (clinical trial registration number NCT00084383)
Pancreatic cancer	Adenoviral vector	human tumor necrosis factor (TNF)-alpha gene regulated under the control of a radiation-inducible gene promoter TNFerade followed by chemoradiation	Early Phase I/II	Tumor shrinkage was observed which facilitates operability to resect the tumor
Pancreatic cancer	Adenoviral vector	TNFERade plus radiation followed by autologous dendritic cells versus radiation only followed by cell vaccine injection	Phase II	Ongoing (clinical trial registration number NCT00868114)
Arm A (for resectable tumors) and Arm B (for locally advanced tumors)	Recombinant adenoviral vector	Expression of the TK gene (AdTK) followed by valacyclovir	Phase I	Ongoing (clinical trial registration number NCT00638612)
Pancreatic cancer	Replication-competent viruses ONYX-15	-	Phase I/II	Very limited replication capacity which compromised its antitumoral effect
Non-metastatic pancreatic adenocarcinoma	Replication competent adenovirus	Combining suicide gene therapy delivered by Ad5-yCD/mutTKSR39rep-ADP with chemoradiation therapy	Phase I	Ongoing (clinical trial registration number NCT00415454)
Unoperable pancreatic cancer	Herpes simplex virus	OncoVEX GM-CSF	Phase I	Ongoing (clinical trial registration number NCT00402025)
Advanced pancreatic cancer	Cholesterol liposome-based nanoparticles	The pro-apoptotic Bik gene	Phase I	Ongoing (clinical trial registration number NCT00968604)
Advanced pancreatic Adinocarcinoma	-	LMB-9 immunotoxin	Phase I	Ongoing (clinical trial registration number NCT00010270)

the limited number of clinical trials indicates difficulty from moving lab to clinic (Table 2). The disseminating nature of pancreatic cancer at the time of diagnosis indicates the need to develop an effective therapy and there are still a few areas that can be improved. One of the great interesting and promising strategies is to develop clinically successful oncolytic viruses and micro RNA based therapy. These studies have provided very encouraging signs that current research is on the right developing path having significant impact on clinical setting. The combination of the developed gene therapy with the existing cytotoxic agent will be more interesting for the development of successful treatments of pancreatic cancer. By incorporating the basic knowledge of genetic targets of the pancreatic cancer, it will become possible to bring a more potent, sophisticated, personalized and selective anti tumoral gene therapy having clinical applicability.

Conclusion

Pancreatic cancer is a belligerent and malignant disease with limited prognosis. All existing therapeutic strategies are relatively ineffective in improving the survival rate except surgical resection which is possible in very small number of patients. The advancements in the cancer gene therapy including the suitable vector, molecular targets and target selectivity as well as detailed understanding of the genetics of the pancreatic cancer, eased the development of new

therapeutic modalities. However, clinical success of the gene therapy is limited due to involvement of the several genes which impedes the complete cure of the disease. By applying multimodality approach through incorporation of newer therapeutic strategies with existing therapies and incorporating the improved delivery vector having targeting potential becomes the more prolific approach, which may further improve the efficacy in treatment of pancreatic cancer.

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