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New Strategies for Cancer Gene Therapy: Progresses and Opportunities

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Abbreviations: herpes simplex virus thymidine kinase (HSV-TK), cytosine deaminase (CD), tumour infiltrating lymphocytes (TIL), interleukin (IL), herpes simplex virus (HSV), interferon (IFN), tumour necrosis factor (TNF), carboxyl esterase (CE), carboxypeptidase A (CPA), nitroreductase (NR), thymidine kinase (TK), multiple-drug activation enzyme (MDAE), phosphatase and tensin homolog (PTEN), breast cancer 1 (BRCA1), tissue inhibitor of metalloproteinases (TIMPS), platelet factor 4 (PF-4), thrombospondin-1 (TSP-1), tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), Adeno-associated virus (AAV), carboxypeptidase G2 (CPG2), FMS-like tyrosine kinase 3 (Flt3),

Summary/Abstract

1. To date, cancer persists as one of the most devastating diseases worldwide. Problems including metastasis and tumour resistance to chemotherapy and radiotherapy have seriously limited therapeutic effects of existing clinical treatments.

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2. To address these problems, cancer gene therapy has been developing over the last two decades, specifically designed to deliver therapeutic genes to treat cancers using vector systems. So far, a number of genes and delivery vehicles have been evaluated and significant progresses made with several gene therapy modalities in clinical trials. However, the lack of an ideal gene delivery system remains a major obstacle for its successful translation to the clinics.
3. Recent understanding of hypoxic and necrotic regions within solid tumours and rapid development of recombinant DNA technology have reignited the idea of using anaerobic bacteria as novel gene delivery systems. These bacterial vectors have unique advantages over other delivery systems and are likely to become the vector of choice for cancer gene therapy in the near future.
4. Meanwhile, complicated tumour patho-physiology and associated metastasis make it hard to rely on a single therapeutic modality for complete tumour eradication. Therefore, the combination of cancer gene therapy with other conventional treatments has become paramount.
5. This review will introduce important cancer gene therapy strategies and major vector systems that have been studied so far with an emphasis on bacteria mediated cancer gene therapy. Additionally, exemplary combined therapies will also be briefly reviewed.

Key words

Solid tumour, cancer gene therapy, therapeutic gene, gene delivery, anaerobic bacteria, angiogenesis

Introduction

At present, cancer is a major cause of morbidity and mortality with significant disease burden in both developing and developed countries. Among different types of cancer, lung, stomach, colorectal, liver, prostate, and breast cancer cause most substantial mortality. Therefore, successful treatment of cancer will have a significant impact on improving patient survival. However, problems such as difficulties in early diagnosis, tumour metastasis, and insufficient specificity of chemotherapeutic drugs and radiation against cancer cells have dramatically impaired the therapeutic effects of major clinical treatments. Novel therapies that overcome shortcomings of current available therapies are in urgent demand. Cancer gene therapy is one of them that has already shown promise as a novel anti-cancer modality.

Cancer gene therapy- progresses and challenges

Gene therapy involves the delivery of therapeutic genetic materials into specific target cells. In 1991, the first clinical trial of cancer gene therapy was performed in melanoma patients (1). In 2000, a clinical trial involving a retroviral vector system for treating severe combined immunodeficiency-x1 patients who suffer from serious combined immunodeficiency achieved complete correction of disease phenotype (2). The trial demonstrated promising therapeutic potentials of gene therapy. To date, a number of strategies have been designed and tested for cancer gene therapy, which can be divided into five main categories including suicide gene therapy, rehabilitation of aberrant cell cycle, immunomodulatory gene therapy, anti-angiogenesis gene therapy, and oncolytic gene therapy (Table 1).

Suicide gene therapy

In suicide gene therapy, also known as gene-directed enzyme prodrug therapy, protein products of therapeutical genes (also known as transgenes) are enzymes that convert non-toxic prodrug into cytotoxic drug such as herpes simplex virus thymidine kinase (HSV-TK) and bacterial cytosine deaminase (CD). For cancer treatment, transgene encoding prodrug converting enzyme is initially delivered to target tumour cells by a vector system. Once the enzyme is expressed within these tumour cells, a non-toxic prodrug is subsequently administered and converted into a cytotoxic drug by the enzyme within target tumour cells, therefore exerting its anti-cancer function (refer to Table 1 for some exemplary suicide genes that have been used so far).

Rehabilitation of aberrant cell cycle

It is now known that loss-of-function mutations of tumour suppressor gene and gain-of-function mutations of oncogene are closely related with oncogenesis. Therefore, cancer gene therapies utilizing strategies focusing on suppression of dominant oncogenes such as *ras* and *bcl-2* and restoration of normal functions of tumour suppressor genes such as *p53* and *p16* in cancer cells have been investigated at both preclinical and clinical levels. Silencing of dominant oncogenes can be achieved on both DNA and RNA level. Inhibition of oncogene mRNA by ribozyme, anti-sense RNA and small interfering RNA have been extensively studied. Meanwhile, antigene oligonucleotides have been studied to block oncogene expression at transcription level. On the other hand, delivery of functional tumour suppressor genes such as wild-type *p53* into tumour cells has also been a subject of investigation for several types of cancer.

Immunomodulatory gene therapy

Cancer cells' ability to evade host's immune surveillance is one of the most important pathological factors in oncogenesis and tumour development. Therefore, it is critical to reactivate and reinforce the patient's immune responses against malignant cells. Immunomodulatory gene therapy has been designed for such purpose. To this end, a number of methods have been studied, such as the use of gene transfer to facilitate a dormant host immune response directed against the tumour. The vector-induced inflammatory/ immune response can also function as an adjuvant to the transduced antigen, leading to local release of cytokines and an influx of antigen presenting cells. Immunomodulatory therapy strategies have the capacity to involve both *ex vivo* and *in vivo* approaches. Earlier studies of adoptive transfer of *ex vivo* expanded tumour infiltrating lymphocytes (TIL) have produced therapeutical results in both melanoma and renal cell cancer. Systemic administration of interleukin-2 (IL-2) has been reported to enhance the activity of TIL in some trials, but it was unfortunately associated with marked toxicity (3). A second approach in preclinical development involves genetic modification of dendritic cells with IL-7 gene. Studies have shown that IL-7 stimulates cytotoxic T-lymphocyte responses and down-regulates tumour production of various immunosuppressive growth factors such as tumour growth factor- β . The main rationale of this approach is to improve the possibility of enlisting the immune system to substantially amplify gene therapy and, thereby enhancing the therapeutic benefits.

Anti-angiogenesis gene therapy

Angiogenesis is a critical and necessary process in tumour growth. All solid tumours undergo angiogenesis that induces subsequent pathological changes including formation of defective leaky vessels, emergence of a heterogeneous tumour cell population, and presence of intratumoural hypoxia/necrosis regions (4). Hence, development of cancer gene therapy focusing on interference with tumour angiogenesis has become an active field of research. In principle, anti-angiogenesis gene therapy involves the suppression of angiogenic factors such as vascular endothelial growth factor or the reinforcement of angiogenic inhibitors such as endostatin.

Oncolytic gene therapy

In oncolytic gene therapy, different kinds of oncolytic vector are used to enter and eradicate different kinds of tumour cells directly. So far, most vectors used are genetically engineered viruses such as herpes simplex virus (HSV) and adenovirus. They are designed to specifically infect tumour cells and destroy them by propagating, producing cytotoxic proteins, and triggering cell lysis. In addition, oncolytic gene therapy using oncolytic bacterial vectors such as *Salmonella* and *Clostridia* vectors has gained an increasing popularity during the last decade (5).

Other strategies

Strategies such as radiosensitization and chemosensitization have also been evaluated. In these strategies, gene therapy is utilized to sensitize cancer cells to chemotherapeutic drugs or radiation to achieve a superior therapeutic effect.

Major vector systems in use

To deliver a transgene into target tumour cells, a vector system is required. To date, three major classes of vectors have been investigated including viral, synthetic, and bacterial systems.

Viral vectors

Hitherto, most cancer gene therapies have been based on viral vectors because of the fact that viruses have evolved with natural ability and mechanisms to transfer their DNA into host cells making them ideal for delivering foreign genetic materials into tumour cells. Currently, virus-mediated cancer gene therapy protocols have used vectors that are mainly developed from retroviruses, HSV, adenovirus, poxvirus, adeno-associated virus (AAV).

Retroviral vectors

Retroviruses are small single-stranded RNA viruses that have the unique ability to replicate in host cells by producing DNA from its RNA genome using enzyme reverse transcriptase. The reversely transcribed DNA is then inserted into host genome and becomes an integral part of host cell genome (6). A wide variety of retrovirus have been identified in many vertebrate hosts such as human, birds and mice (6). Retroviral vectors have been developed by replacing three viral genes, *gag*, *pol* and *env* with various transgenes. Currently, the most frequently used retroviral vectors are derived from the Moloney murine leukemia virus. The major advantage of these vectors is their ability of long-term integration into host cell genome. As a result, transferred therapeutic genes can be expressed throughout the entire life of host cells. In addition, they have a remarkable safety profile as most of their genes encoding viral proteins have been replaced with transgenes. The major defects of these vectors are their inability to transduce non-dividing cells and poor tumour penetration. Furthermore, their stable host cell genome integration might cause activation of oncogenes or silencing of tumour suppressor genes, which is known as insertional mutagenesis. Although efforts have been made to optimise these vectors such as the development of replication-competent retroviral vectors, their inability to transduce non-dividing cells still poses a remarkable challenge. However, the development of lentiviral vector has given new hopes for retroviral vector mediated cancer gene therapy. Lentivirus such as human immunodeficiency virus is a subclass of retrovirus that is involved in the development of diseases characterized by acquired host immunodeficiency (7). Lentiviral vector is a class of complicated retroviral vectors, which is characterized by a highly improved cell transduction efficiency and a large loading capacity for transgenes (7). More importantly, as compared to other retroviral vectors, lentiviral vector is competent in transducing non-proliferating cells as well (7).

Adenoviral vectors

This class of viral vectors have a distinguished ability to transfer foreign genes into a broad range of cells including both dividing and non-dividing cells (8). Adenoviruses replicate episomally and do not insert their DNA into host cell genome, therefore chances of inducing host cell insertional mutagenesis are much less (8). As a result, their gene expression within host cells can only be transient, which is not ideal for treating chronic diseases. There are two groups of genes within adenoviruses: *cis*-genes and *trans*-genes. While the *cis*-genes are essential for viral replication, the *trans*-genes can be entirely replaced with therapeutic genes. Originally, adenoviruses

were modified with either their *E1* or both *E1* and *E3* genes replaced with transgenes. However, these vectors induced some unfavourable host cellular immune responses (9). Subsequently, more genes of adenoviruses were deleted to reduce their immunogenicity (8). The newest generation of adenoviral vectors with low level of immunogenicity and ability of long-term gene expression in host cells have been created. They are produced with almost all their viral genes deleted (both *cis*-genes and *trans*-genes) except the packaging signal. The functionality of these vectors is dependent on the involvement of a helper virus that possesses all vital viral genes for replication is necessary (8).

HSV vectors

HSV is a neurotropic virus, which is known for its ability to infect nerve cells and cause a subsequent latent infection (10). There are two serotypes of HSV: type 1 and 2. Between them, HSV-1 has been mostly studied for cancer gene therapy. HSV vectors have been produced by deleting viral genes encoding viral proteins expressed during early stage of infection such as *ICP27*, *ICP4* and *ICP0* (10). Compared to other viral vectors, HSV vectors have several advantages including the ability to transduce non-dividing cells, robust intratumoural growth, and a large capacity for transgenes (10). The major limitations of HSV vector include the viral nature of transient gene expression and vector induced cytotoxicity.

AAV vectors

AAVs are single stranded DNA viruses that belong to the parvovirus family (11). AAV only replicates in presence of a helper virus such as adenovirus (12). In the absence of a helper virus, AAV integrates its genome into chromosome 19 in human cells establishing a latent cycle (12). Among various serotypes, AAV serotype 2 has been well studied for cancer gene therapy (12). AAV induces negligible host immune responses and is able to infect non-dividing cells making it a promising vector system (12). To produce recombinant AAV vector, two viral genes, *Rep* and *Cap* have been replaced with transgenes. The major shortcoming of AAV vectors is their limited transgene loading space.

Poxviral vectors

Poxviruses are a family of complex double stranded DNA viruses. These viruses contain a large genome and replicate in host cell cytoplasm. For gene therapy, an attenuated poxvirus, vaccinia virus that was originally used as a vaccine against smallpox, has been extensively studied. Poxviruses can not only be engineered to

transfer transgenes into target cancer cells but induce oncolysis as well. To date, most of poxviral vector applications have been limited to immunomodulatory strategy (13). The major advantages of poxviruses are their low toxicity, large loading capacity for transgenes, high level of transgene expression, and the ability to transduce both dividing and non-dividing cells.

Oncolytic viral vectors

In the case of oncolytic viral therapy, oncolytic viruses are genetically modified to replicate selectively within tumour cells. To increase their specificity against cancer cells, oncolytic viruses are engineered to only replicate in actively dividing cells like cancer cells. As a result, these viral vectors are tumour-specific, self-replicating, and oncolytic cancer killers. Currently, many viruses have been used for oncolytic viral therapy such as adenovirus, HSV-1, poxvirus, measles, and newcastle disease virus (13). Although these vectors have displayed promising potentials, they also share a number of drawbacks with other viral vectors such as biosafety risks and induction of host immune responses.

Although significant progresses have been made during previous years, stumbling blocks still exist in viral cancer gene therapy. Problems such as insufficient vector specificity to cancer cells, incomplete vector transduction of tumour cells, dissemination of vector to normal organs, and vector induced host immune responses have to be solved for their further development.

Synthetic vectors

Generally, synthetic vectors are not as efficient as viral and bacterial vectors in gene delivery and expression. However, they do have a number of advantages. For example, these vectors have the unique ability of delivering therapeutic agents such as chemotherapeutic drugs and synthetic oligonucleotides to tumour cells (14). Currently, synthetic vectors are mainly produced from liposome and polymers (14). Recently, synthetic semiconductor quantum dots have been tested in several studies and showed promising potentials for targeted anti-cancer treatment and clinical detection of primary and metastatic tumours (15).

Bacterial vectors

Hypoxia in solid Tumours has caused many problems in cancer treatment with recent studies shown that it plays a pivotal role in tumour angiogenesis, development, and metastasis. Severe hypoxia in tumours has been directly linked to poor prognosis in patients. In addition, it has also been found to be responsible for the resistance of

tumour cells against radiotherapy and chemotherapy. Although most viral vectors display low transduction efficiency against hypoxic tumour cells, the hypoxic and necrotic regions in the tumours provides an ideal habitat for anaerobic bacteria and an excellent opportunity for the development of anaerobic bacteria-mediated cancer gene therapy. Actually, the history of treating cancer using live bacteria dates back over a century. In 1813, the apparent recovery of a cancer patient, who was concurrently suffering from gas gangrene caused by *Clostridium* infection, from cancer was reported (5). The finding stimulated extensive interests in treating cancer using live bacteria or bacterial toxins (5). Predominantly, three classes of anaerobic bacteria have been studied for cancer treatment: Bifidobacteria, Salmonella, and Clostridia. Although studies have shown that these bacteria were highly selective and preferentially colonised within solid tumours and did cause tumour regression in several cases, complete tumour eradication was never reported from human patients (5). Nevertheless, recent advances in recombinant DNA technology and the finding that regions of hypoxia and necrosis are consistently present within solid tumours have prompted the idea of using genetically engineered anaerobic bacteria as vectors for cancer gene therapy.

Bifidobacterial vectors

Initial studies showed that Bifidobacteria administered into tumour bearing mouse colonised mainly within tumours with almost no bacteria detected in other organs. In 1999, a study using *B. adolescentis* demonstrated apparent inhibition of occurrence and development of colorectal carcinoma and induced tumour apoptosis *in vivo* (16). Strategies used for Bifidobacteria mediated cancer gene therapy can be divided into 4 groups including tumour suppressor gene delivery, suicide gene therapy, anti-angiogenesis, and chemosensitization. Among various strains of Bifidobacteria, *B. longum* has been mostly studied. For tumour suppressor gene therapy, human Phosphatase and tensin homolog (PTEN) gene was successfully introduced into *B. longum* and the recombinant bacteria resulted in obvious inhibition on growth of mice solid tumours (17). To evaluate the efficiency of suicide gene therapy, CD gene was introduced into *B. longum* and *B. breve* (18). In addition, approaches aimed at interfering with tumour angiogenesis has also been carried out. Both *B. longum* and *B. adolescentis* were modified to express endostatin, which is a tumour angiogenesis inhibitor. The results showed that both recombinant bifidobacterial strains induced inhibition of tumour angiogenesis and tumour growth (19, 20). The main advantage

of Bifidobacteria as a vector system is that they are common flora of human intestine imposing low risks of introducing bacterial related disease. Furthermore, they can be administered both orally and intravenously (5). However, they do suffer from several drawbacks. Firstly, Bifidobacteria do not form spores therefore, they are more vulnerable to rigorous conditions and more difficult to handle and store. Secondly, their tumour colonisation efficiency is comparatively low and they tend to clump instead of spreading out within tumours leading to insufficient expression of transgenes within tumours (5). Thirdly, Bifidobacteria are not oncolytic.

Salmonella vectors

Although tumour colonisation property of Salmonella has been known for decades, the bacterial pathogenic characteristics have prevented them from being further exploited as a gene delivery system. Fortunately, recent advances in recombinant DNA technology have enabled the creation of a novel attenuated non-virulent *Salmonella* strain, *S. typhimurium*, with the original *purI* and *msbB* gene of the bacteria deleted (21). In 1997, attenuated *S. typhimurium* was, for the first time, studied for anti-cancer purpose (21). The results showed the attenuated bacteria retained excellent tumour colonisation ability, whilst their pathogenicity was efficiently limited. More importantly, intraperitoneal injected microbe successfully suppressed tumour growth and significantly prolonged average survival of B16F10 melanoma tumour bearing mice (21).

Attenuated *S. typhimurium* has also been studied in the context of suicide gene therapy. When attenuated *S. typhimurium* was initially evaluated for its anti-cancer potentials, researchers also engineered it to produce HSV-TK. The administration of the recombinant bacteria successfully expressed the prodrug enzyme in target tumour cells and phosphorylated prodrug ganciclovir into its toxic form (21). In another study, attenuated *S. typhimurium*, VNP20009, was genetically modified to produce prodrug-activating enzyme carboxypeptidase G2 (CPG2) (22). The results showed that administration of CPG2-expressing bacteria alone did inhibit the tumour growth. However, when various prodrugs activated by CPG2 were jointly administered with CPG2-expressing bacteria, bacteria-induced oncolytic effect was significantly improved. In addition to suicide gene therapy, Salmonella has also been studied for immunomodulatory gene therapy. In three separate studies, attenuated *S. typhimurium* was engineered to express IL-2 in order to stimulate hosts' immune responses against liver cancer, neuroblastoma, and melanoma (23-25). In all three studies, IL2-

expressing *S. typhimurium* successfully resulted in inhibition of tumour growth. Furthermore, one of the studies showed increased numbers of hepatic and circulating NK1.1(+)CD3(-) lymphocytes in experimental mice (23) and another study confirmed that the anti-tumour response was correlated with decreased tumour angiogenesis and increased tumour apoptosis (25). In a different study, Yoon and colleagues produced a genetically engineered *S. typhimurium* containing FMS-like tyrosine kinase 3 (Flt3) ligand expression vector (26). The Flt3 ligand is a small molecule operating as a growth factor that increases the number of immune cells by activating the hemtopoietic progenitors (26). The results showed that recombinant Salmonella induced inhibition of tumour growth and extended the survival of melanoma tumour-bearing mouse.

Besides *S. typhimurium*, another attenuated Salmonella strain, *S. choleraesuis*, has also been produced. Cancer gene therapy involving attenuated *S. choleraesuis* has mainly been focused on anti-angiogenesis and augmenting the therapeutic effects of chemotherapeutic drugs. One of the major drawbacks of Salmonella mediated cancer gene therapy is that their tumour to normal tissue colonisation ratio is comparatively high as a result of the microbe being a facultative anaerobe (5). To further increase its specificity to solid tumours, an attenuated auxotrophic Salmonella strain, *S. typhimurium* A1, was produced by Zhao and Colleagues (27). They confirmed that the novel attenuated *S. typhimurium* A1 was able to induce tumour growth retardation by not only growing in viable tumour tissues, but in hypoxic and necrotic intratumoural sites as well, whereas normal non-tumour tissues were cleared of these bacteria (27). To date, two unsuccessful phase I clinical trials using attenuated *S. typhimurium* have been carried out (28, 29). The unsatisfactory results from these two trials were thought to be caused by insufficient bacterial tumour colonisation. The results indicated that there are still considerable works to be done before Salmonella mediated cancer gene therapy will be finally ready for clinical use. The main advantage of Salmonella lies in their ability to grow in both viable and quiescent tumour cells. Hence, theoretically all cancer cells within a solid tumour will be therapeutically interfered in the context of sufficient tumour microbial colonisation.

Clostridial vectors

The innate properties of tumour colonisation and oncolysis of Clostridium have been known for many years. In 1947, Parker and colleagues deliberately injected *C. histolyticum* spores into transplanted mouse sarcomas. The results showed Clostridia

induced tumour oncolysis and regression (5). Later, the isolation of a non-pathogenic proteolytic strain, *C. butyricum* M-55, triggered more extensive studies. In all these studies, clostridial oncolysis was constantly observed and induced the destruction of large parts of tumours. Unfortunately, since outer rim of solid tumour has sufficient blood supplies, it consistently remained unaffected by anaerobic microbial invasion leading to subsequent tumour regrowth (5). Due to the fact that clostridial spores alone can not efficiently eradicate all tumour cells, researchers started to evaluate the therapeutic effect of combined therapy. Administration of clostridial spores has been combined with other therapeutic agents such as radiation. A number of co-administered treatments such as radiofrequency therapy, local X-irradiation, and decreasing the oxygen concentration in the air that tumour-bearing animals breathe has helped to enhance clostridial oncolysis, however, unsatisfactory results from clinical trials finally led to temporary cessation of research in this field (5). In 2001, Dang and colleagues screened 26 species of commonly used anaerobic bacteria and found a strain of Clostridia, *C. novyi*, possessing the best tumour colonisation ability (30). Subsequently, they created a novel strain *C. novyi-NT* by deleting the gene coding for lethal toxin of *C. novyi*. While administration of *C. novyi-NT* alone did not induce significant therapeutic results, combination therapy of *C. novyi-NT* and traditional chemotherapeutic drugs resulted in a significant tumour control. However, due to the extensive tumour lysis, experimental animals died of severe systemic toxicities (30). In a subsequent study performed by the same research group using the combination of *C. novyi-NT* with anti-microtubule chemotherapeutic drugs successfully caused tumour regression of several mouse xenograft tumours without inducing fatal systemic toxicity (31). Meanwhile, clostridial based cancer gene therapy has also been initiated due to the rapid development of recombinant DNA technology. Strategies for clostridial cancer gene therapy are mainly tumour suppressor gene therapy and suicide gene therapy. In the case of tumour suppressor gene therapy, clostridial strains have been genetically engineered to produce cytokine IL2 and tumour necrosis factor alpha (TNF α) (32, 33). Moreover, these strains were also genetically modified to express various prodrug converting enzymes such as CD and nitroreductase (NR) (34). Unfortunately, these genetically modified Clostridia failed to show sufficient therapeutic effects, most likely because of their relatively low tumour colonisation efficiency. Indeed, all these genetically modified strains were saccharolytic Clostridia whose tumour colonisation levels are almost 1000-fold lower

than their proteolytic counterparts (5). Earlier studies were hampered due to the lack of an efficient genetic modification protocol for proteolytic clostridial strains. Fortunately, in 2006, a protocol using conjugation for modifying proteolytic Clostridia was developed (35). As a result, proteolytic clostridial strains with superior tumour colonisation ability such as *C. novyi-NT* and *C. sordellii* are now available for cancer gene therapy studies. Compared to viral vectors, clostridial vectors have several advantages including less biosafety concerns, poor immunogenicity, ease of administration, no requirement for repetitive administrations, eradication of a wide range of intratumoural cells, flourishing growth within hypoxic tumour microenvironment, higher specificity toward solid tumours, no risk of inducing insertional mutagenesis, and a huge loading capacity of transgenes.

Multiple modality synergies

Tumour angiogenesis, hypoxic tumour microenvironment, heterogeneous tumour cell population, and increased tumour interstitial pressure are the major hurdles in successful cure of solid tumours. These multiple challenges make it quite clear that the possibility of completely eradicating all malignant tumours including both primary and metastatic ones by single therapeutic modality is extremely low. Earlier experiences showed us that therapeutic effects of cancer gene therapy can be enhanced by combining with other anti-cancer modalities. As a result, more and more combined therapy has been extensively investigated. Currently, cancer gene therapy has been mainly combined with radiotherapy and chemotherapy. There are two scenarios in these combinations. In one situation, radiotherapy and chemotherapy are used to enhance the therapeutic effect of gene therapy. For example, it was found the combination of an anti-angiogenic gene therapy and low dose radiotherapy was an efficient and externally controlled cytotoxic system (36). In the other instance, gene therapy is used to intensify the therapeutic effect of radiotherapy and chemotherapy. For example, the delivery of adenoviral E1a gene helped to sensitize cancer cells to a mitotic inhibitor, paclitaxel (37).

Besides radiotherapy and chemotherapy, gene therapies employing different anti-cancer strategies can also be combined with one another to achieve synergic effects. In one of the studies, delivery of both second mitochondria-derived activator of caspases and TRAIL by engineered *S. typhimurium* resulted in significant synergic antitumoural effects (38).

Our laboratory has been working on clostridial mediated cancer gene therapy. We are currently engineering the *C. novyi-NT* to express tumour-specific immunotoxins, with an aim to induce enhanced oncolysis within primary solid tumours and large metastatic tumours. To date, such immunotoxins have been mainly evaluated on leukemia and lymphoma and showed promising therapeutic effects. With respect to solid tumours, such studies have been only executed by intratumoural injection, which is not ideal for clinical application.

Conclusion

Complete cure of cancer has never been an easy task. The awful ability of malignant tumours to survive various therapies, their property of evading host immune system, and tendency of metastasis are major challenges in cancer treatment. To address these challenges, researchers have invested substantial efforts to create novel potent anti-cancer therapies. The emergence of cancer gene therapy has brought us new hopes. However, the lack of an ideal gene delivery system has been a major roadblock. Recently, the development of novel self-replicating, tumour targeting, oncolytic viral vectors and targeted synthetic vectors has displayed significantly improved therapeutic effects. The rapidly growing field of anaerobic oncolytic bacterial mediated cancer gene therapy has also shown the potential to stimulate a quantum leap in cancer treatment. By combining various anti-cancer modalities together, it is possible to envisage a therapeutic cure for many kinds of malignant tumour.

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Table 1 Strategies and exemplary transgenes used for cancer gene therapy

Strategy	Transgenes	Reference
Immunomodulatory gene therapy	IFN- γ , IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, TNF- α	(10)
Suicide gene therapy	CE, CPA, CD, NR, TK, MDAE, Cytochrome P450	(5)
Rehabilitation of aberrant cell cycle	p53, p21, p16, p73, PTEN, BRCA1, E2F-1	(39)
Anti-angiogenesis	P53, TNF- α , TIMP-1,2,3, Endostatin, Angiostatin, PF-4, IL-12, TSP-1	(16)
Gene therapy combined with chemotherapy	p53, TRAIL, E1a, cytochrome P450	(40)
Gene therapy Combined with radiotherapy	p53, E2F-1, TNF- α , PTEN	(41-43)

Interferon (IFN), Interleukin (IL), Tumour necrosis factor (TNF), Carboxyl esterase (CE), Carboxypeptidase A (CPA), Cytosine deaminase (CD), Nitroreductase (NR), Thymidine kinase (TK), Multiple-drug activation enzyme (MDAE), Phosphatase and tensin homolog (PTEN), Breast cancer 1 (BRCA1), Tissue inhibitor of metalloproteinase (TIMP), Platelet factor 4 (PF-4), Thrombospondin-1 (TSP-1), Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)