Review Article

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Suicide gene therapy: A special focus on progress and concerns about cancer treatment

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Abstract

Suicide gene therapy is based on transfer of a suicide viral or a bacterial gene into tumor cells. Expression of these genes leads to non-toxic substances in cell that are converted to lethal chemotherapeutic agents. Although this approach to gene therapy has been widely welcomed by gene researchers and successfully used in many *in vivo* and *in vitro* researches, in the case of human clinical trials, the results were not repeated and did not have appropriate clinical consequences. Nevertheless, current studies on preclinical models of cancer revealed that suicide gene therapy has a high potential when used in combination with novel therapeutic strategies. This review article summarized various types of suicide gene strategies as well as particular highlighting of the recently developed suicide gene therapy in humans and their adverse effects. Moreover, the diverse systems which have been used along with suicide gene therapy were reviewed. Finally, this article provides some perspectives into the future of this approach, particularly for eradication of tumor stem cells.

Keywords: Bystander effect, Cancer clinical trials, Prodrug therapy, Suicide gene therapy, Tumor cells.

1. Introduction

Cancer, the second leading cause of death worldwide, has emerged as a major public health problem worldwide, so that approximately 1/6 of global deaths were due to cancer in 2016 (1). The classical approaches for treating various types of cancers are surgery, chemotherapy, and radiotherapy. If cancer cells are diagnosed in the early stages, complete surgical resection is effective; but, tumors often are detected in the advanced stages and the possibilities of surgery are limited (2). Furthermore, recent studies showed that many tumors display intracellular heterogeneity with subsets of cancer stem cells that sustain the growth and recurrence of tumor cells. Therefore, it helps to resistance against conventional therapeutic approaches and emerge a new challenge for cancer treatment (3). Moreover, current systemic therapies could cause several life limiting adverse effects, which creates the need of dose reduction for chemotherapeutics agent or radiation below the most effective levels. Sum of these problems together with other technical difficulties demonstrate the necessity for development of more efficient cancer therapy strategies (3).

A promising possibility for cancer therapy is the technologies that transfer genetic material to tumor cells. The emerging field of cancer gene therapy proposes a series of sensational ways for cancer therapy, which have been recently evidenced by the successful clinical trials. The term "gene therapy" includes a wide range of therapeutic approaches that involves the introduction of new genetic materials into a series of cells, to retrieve or improve gene expression, in order to treat the disease. In the current century, many in-vitro and preclinical animal studies investigated a

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variety of gene therapy strategies to treat cancer, including induction of apoptosis, oncolytic virotherapy, stimulation of the immune system, anti-angiogenic gene therapy, correction of gene defects, inhibition of tumor invasion, gene therapy to enhance chemo- and radiotherapy, myeloprotective gene therapy, antisense and RNA interference (RNAi) based strategies, and pro-drug activation/ suicide gene therapy (4). Among them, suicide gene therapy has attracted a lot of attention due to employing a rational way for introduction of a gene that can directly (or by activation of a prodrug) suppresses the tumor growth into cells.

The present review will study and discuss suicide gene therapy strategies that are currently applied for cancer therapy, with a brief review on different suicide gene systems and gene delivery vectors, and a particular focus on the latest approaches used to optimize their efficacies.

2. What is the suicide gene therapy?

The principle of suicide gene therapy is delivery of a transgene encoding for a non-mammalian protein into the tumor cells, which allows the conversion of a subsequently-administered inactive prodrug into its active form, evoking the death of cells that express the therapeutic gene. Sometimes, the product of introduced gene can be a toxic protein that results in cell death. Accordingly, three possible approaches can be used for suicide gene therapy: (a) indirect tumor targeting by introduction of a prodrug activating suicide gene (Figure 1.a), (b) direct tumor targeting by using toxin gene (Figure 1.b), and (c) introduction of pro-apoptotic genes (e.g. tumor suppressor genes) (Figure 1.c).

2.1. Approach (a): introduction of a prodrug activating suicide gene

This strategy is based on the fact that some non-mammalian organisms (e.g. bacteria, viruses, and even fungi) use metabolic systems that do not exist in mammalian cells. A number of antibiotics also relies on these unique metabolic pathways. Therein, invading microorganism converts the antibiotic precursor (or prodrug) to the active form using acquired enzymatic digestion. After invasion, the active antibiotic is only formed in the infected cells where in the invading microorganism produce the required enzyme. The products of these enzymatic processes inhibit the growth of (or kill) the pathogenic microorganism, while are not harmful for human cells due to the lack of such enzymatic systems (5). The principle of prodrug activating suicide gene therapy for cancer is based on the transfection of microbial enzymatic genes that have the ability to convert a non-toxic prodrug into a cytotoxic drug. After administration, the non-toxic prodrug is broadly distributed thorough the body, but is only activated in the tumor cells (6).

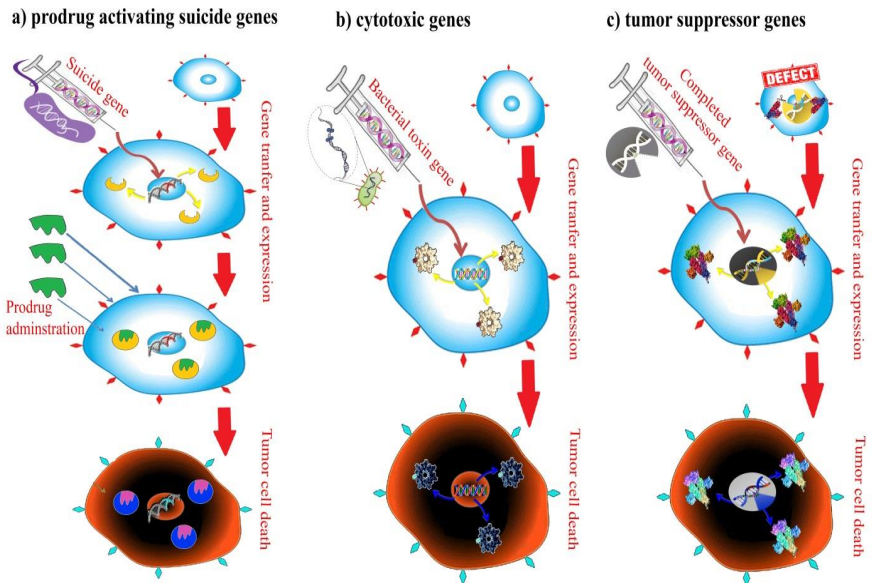

Figure 1. Three different approaches commonly used for cancer suicide gene therapy.

Table 1. Different systems using prodrug-activating suicide gene therapy approach.

Prodrug	Gene encoded enzyme	Origin of gene	Active drug	Bystander effect
5-Fluorocytosine	Cytosine deaminase	*E. coli*, Yeast	5-Fluorouracil	√
6-Methylpurine deoxy riboside	Purine nucleoside phosphorylase	*E. coli*	6-Methylpurine	√
Ganciclovir	Thymidine kinase	Herpes simplex virus	Ganciclovir triphosphate	√
6-Methoxypurine arabinoside	Thymidine kinase	Varicella-Zoster virus	Adenine arabinoside triphosphate	√
Cyclophosphamide	Cytochrome P450-2B1	Rat	Phosphoramide mustard	√
Ifosfamide	Cytochrome P450-2B1	Rat	acrolein	√
Cephalosporin derivatives of doxorubicin	Beta-lactamase	Bacterial	Doxorubicin	√
Carboxybutanamido cephalosporin mustard	Beta-lactamase	Bacterial	phenylenediamine mustard	NR[1]
Chloroethyl Mesyloxy ethyl amino benzoyl-L-glutamic Acid	Carboxypeptidase G2	Pseudomonas RS16	Chloroethyl mesyloxyethyl amino benzoic acid	NR
6-Thioguanine	Guanosine-xanthine phosphoribosyl transferase	*E. coli*	6-Thioguanine triphosphate	√
β-D-galactopyranosyl nitrobenzyloxycarbonyl daunomycin	β –Galactosidase	*E. coli*	Daunorubicin	√
Methotrexate -α-peptides	Carboxypeptidase A	Rat	Methotrexate	NR
2-Aminoanthracene	Cytochrome p450-4B1	Rabbit	DNA-alkylating agents	√
Acetaminophen	Cytochrome p450-4B1	Rabbit	N-acetyl bezoquinone imine	√
Linamarin	Linamarase (β -glucosidase)	plant Cassava	Acetone cyanohydrin	√
Indole acetic acid	Peroxidase	plant Horseradish	Free radicals	NR
Aziridinyl dinitrobenzamide (CB1954)	Nitroreductase	*E. coli*	Aziridinyl hydroxylamine nitrobenzamide	√
Acetylated 6-Thioguanine, Methotrexate, and other Purines	Multiple drug activating enzyme	Plant Tomato	6-Thioguanine, Methotrexate, cytotoxic purines	√

[1]NR: Not reported

Among the three predefined approaches, suicide gene therapy has been widely investigated and various suicide systems using this strategy have been developed until now (Table 1). Though many suicide systems have been designed, only a few number were generally accepted. Among them, the systems based on "*Escherichia coli* cytosine deaminase with 5-fluorocytosine", "Varicella-Zoster

thymidine kinase with 6-methoxypurine arabinonucleoside", "Herpes simplex thymidine kinase gene with ganciclovir" are the most extensively studied. However, in recent years, some other systems have been developed based on "*Escherichia coli* nitroreductase with aziridinyl dinitrobenzamide", "*Escherichia coli* gtp gene with 6-thioxanthine", and "*Escherichia coli* deo gene and 6-methyl purine deoxyriboside" (5). In addition, some systems employ the conversion of anticancer prodrug (cyclophosphamide and ifosfamide) by human P450 isoforms into potent anticancer metabolites (phosphoramide mustard and acrolein) (7).

One of the most repeatedly reported therapeutic systems is based on the transfection of cytosine deaminase gene (CDase) of *Escherichia coli* together with administration of 5- fluorocytosine (5-FC), a non-toxic antifungal agent, as a prodrug. CDase, a fungal or bacterial enzyme, catalyzes hydrolytic deamination of cytosine to form uracil, and 5-FC to 5-fluorouracil (5-FU), and essentially involves in the metabolism of pyrimidine (8). Since CDase is present in bacteria and fungi, but not in normal human cells, the gene encoding CDase has been exploited in an enzyme/prodrug gene therapy approach for treatment of cancer. CDase is selectively delivered to tumor cells; and then, the patients took the approximately nontoxic 5-FC prodrug. After deamination by CDase, 5-FU is converted to 5-FdUMP, 5-FdUTP, or 5-FUTP (e.g. potent pyrimidine antimetabolites) by intracellular enzymes that are irreversible inhibitors of thymidylate synthase, thereby preventing DNA replication by blocking deoxy thymidine triphosphate (dTTP) synthesis, a widely used antitumor agent. Moreover, these metabolites formed (5-FU) RNA and (5-FU) DNA complexes, which interfere in RNA and DNA synthesis and finally lead to induction of cell death (9, 10). In addition, there are some reports that declared apoptosis also plays an important role in the mechanism of cytotoxicity induced by the 5-FC (11). Mitochondrial pathway is also reported, which is involved in the process of cell death induced by 5-FC suicide system, while p53 and death receptors are not implicated in such process (11). All these metabolic conversion occurs selectively in the gene-delivered tumor cells and allowed to localize general toxicity without dependency to active transport. Nevertheless, the CDase/5-FC system relies on the bystander effect for tumor ablation (12).

Additionally, a new variant of CDase gene derived from Candida kefyr was designed to enhance the capability of 5-FC conversion in comparison to the *E. coli* one (13). The Candida kefyr CDase is a more potent enzyme, which enhances the cell sensitivity to 5-FC as well as increases the bystander effect previously seen in CDase/5-FC system.

Another most promising suicide gene therapy system is based on the viral thymidine kinase gene (TKase) and antiviral agent ganciclovir (GCV). GCV, years ago, was developed as an antihepatitis medicine derivated from acyclovir and considered as a low-toxic drug for mammalian cell, due to the need to become monophosphrylate by thymidine kinase enzyme (14). Mammalian thymidine kinase is a weak enzyme for catalyzing this process, while, Herpes simplex virus type 1 produces a type of thymidine kinase enzyme that does not have similarity with mammalian enzyme. This enzyme is more potent in activating GCV than the mammalian cellular kinase (15). In TKase/GCV system, the expression of the Herpes simplex virus thymidine kinase gene (HSV-TKase), which leads to the production of viral thymidine kinase, converts GCV to GCV monophosphate and then GCV triphosphate. GCV triphosphate as an analogue of deoxyguanosine triphosphate can inhibit the enzyme DNA polymerase and disrupt the DNA synthesis process, which finally induce apoptosis and cell death (11). However, the studies showed that this apoptosis was not occurred in a direct chemical way. The anticancer mechanism of TKase/GCV system is delayed in the S phase and hence, G2- phase arrest by the activation of exonuclease. In fact, the apoptosis observed in this system is due to accumulation of p53, translocation of CD95 to the cell surface and formation of a death-inducing signaling complex containing Fas-associated death domain protein (FADD) and caspase-8, in addition to the extensive destruction of mitochondria (16, 17). Similarly to CDase/5-FU, if HSV-TKase is introduced to tumor cells, the conversion is specially occurred by cancer cells'

enzymes; therefore, the cytotoxicity of agent will be targeted.

Another viral thymidine kinase gene was also used for suicide gene therapy. Varicella-zoster virus thymidine kinase (VZV-TKase) gene is one of the oldest suicide genes applied for genetically tumor cell therapy (18, 19). In several studies, 6-methoxypurine arabinonucleoside (ara-M) was considered as a prodrug (20-22). The process of activation of ara-M (phosphorylation) was strongly catalyzed by VZV-TKase. The next step of phosphorylation was physiologically occured by four cellular proteins: AMP deaminase, adenylosuccinate synthetase lyase, AMP kinase, and nucleoside diphosphate kinase (23). As usual, since VZV-TK gene are transduced to mammalian cells, following the administration of ara-M cell apoptosis and cell death is induced.

2.1.1. Bystander effect

Despite the development of numerous vectors in gene therapy, transfection efficacy of current gene delivery systems is usually low, which results in low expression profile. An amazing phenomena that greatly increases the efficacies of cancer cell therapy by suicide gene delivery is "bystander effects". It should be noted that the enzyme produced by a type of cells affects not only themselves, but also the adjacent cells and tissues. Therefore, in suicide gene therapy, the therapeutic results may spread outside the transfected cancer cells and despite un-transfection, surroundings of the tumor lesions could regress by this therapy. This phenomenon is known as bystander effect that may more or less retrieve the low efficacy of gene transferring to tumor cells. Due to presence of this phenomenon, complete tumor regression has been indicated after prodrug administration even when only 10% of the cancer cells are transduced by suicide genes (24, 25). Up to now, at least five mechanisms have been investigated for bystander effect: (1) release of activated soluble toxic factors, (2) passive diffusion , (3) transferring through gap junctions, (4) phagocytosis of apoptotic molecules, and (5) stimulation of immune response in tumor microenvironment (26-31).

Bystander effect is one of the most important aspects of suicide therapy and nearly all systems using this approach benefit from this phenomenon. In fact, the bystander effect is necessary for the therapeutic success of suicide gene therapy. 5-FU is a chemotherapeutic agent that can be transported out of the cells by simple diffusion; therefore, a strong local bystander effect was occurred in the CDase/5-FC system without need for cell-to-cell contact (32, 33). In this system, only 1-30% of the transfected cells are sufficient to prevent the growth of the untransfected adjacent cells *in vitro* (33). Clinical and in-vivo studies have been reported even better results (34-36).

The unmodified neighboring cells near to TKase-expressing cells are also affected by apoptotic bodies generated from transfected cells that are uptaken by endocytosis (37). Though the simple diffusion of GCV is low, the TKase/GCV system also benefits from the bystander effect (38). In addition, there are some reports that showed the bystander effect mediated by soluble factors was responsible to phosphorylate GCV metabolites in untransfected cells (39). Moreover, regression of untransduced cancer cells growing at a distance from the transduced tumors is well documented in the TKase/GCV system duo to the induction of immune T lymphocyte response to tumor cells by the transduced cells (40, 41).

2.2. Approach (b): introduction of the genes encoding a cytotoxic protein

The strategy (b) will be taken when the genetic sequence of an effective bacterial toxin are available. In this approach, the gene encoding a cytotoxic protein is selectively targeted to tumor cells, and the product acts as a chemotherapeutic agent. This occurrence is happening only inside the tumor with the help of viral or bacterial suicide inducing genes, and there is no impact on normal cells. The main advantage of this approach is the fact that it doesn't need a prodrug administration and, hence, the prodrug-related problems such as toxicity of metabolites and limited bioavailability was resolved.

In the current century, scientists more and more facilitated the processing and manipulation of biological molecules, among which are bacterial toxins and their encoding genes. Several microorganisms naturally produce toxic proteins that

Table 2. Some bacterial toxins and their activities.

Bacterial toxin	Bacterial Source	Activity	Intracellular target
Diphtheria toxin	*Corynebacterium diphtheria*	ADP-ribosyl transferase	Elongation Factor 2
Pseudomonas Exotoxin A	*Pseudomonas aeruginosa*	ADP-ribosyl transferase	Elongation Factor 2
Anthrax toxin	*Bacillus anthracis*	Adenylyl cyclase	cAMP-induced changed of protein
Anthrax toxin	*Bacillus anthracis*	Zinc endoprotease	MAPKK
Clostridium botulinum C2 toxin	*Clostridium botulinum*	ADP-ribosyl transferase	G-actin
Shiga toxin	*Escherichia coli*	N-glycosidase	28s rRNA
Cholera toxin	*Vibrio cholera*	ADP-ribosyl transferase	Heterotrimeric G-protein
Heat-labile enterotoxin	*Escherichia coli*	ADP-ribosyl transferase	Heterotrimeric G-protein
streptolysin O	Most strains of beta-hemolytic group *A streptococci*	Forming rings and arcs that penetrate into the apolar domain of the bilayer	Cholesterol-containing membranes
Clostridium perfringens enterotoxin	*Clostridium perfringens*	Plasma membrane permeability alterations	Claudin tight-junction protein family
MazF-MazE interferase toxin	*Escherichia coli*	Sequence-specific endoribonuclease	mRNA

target human intracellular moieties. Table 2 shows some of these toxins and their activities. These toxins have the potential to be used in targeted suicide gene therapy systems. Moreover, from many years ago upto now, immunotoxins (hybrid proteins containing a bacterial toxin and an immunoglobulin that binds specifically to target cells) have been extensively applied in cancer treatment (42-45).

From this point of view, the most studied bacterial polypeptide is diphtheria toxin (DT) that was originally obtained from Corynebacterium diphtheria. This cytotoxic two subunit polypeptide can bind to cell membrane and enter to the cytosol via endosomal crossing (46). In the cytosol, DT transfers NAD^+ to a diphthamide residue in eukaryotic elongation factor 2 (eEF-2) (which is needed for protein translation) and blocks the cellular protein synthesis (47). The final consequence of DT is apoptosis and cell death. Thus, if a specific promoter that can selectively target tumor cells was applied, the gene encoding this attractive protein would be served for cancer gene therapy. Using this strategy, Peng, *et al*. designed an adenoviral vector that delivered DNA encoding human prostate-specific antigen (PSA) promoter and DT-A (48). They showed that this transcriptional/DNA recombination control strategy successfully stimulates DT-A expression in a similar manner, which correlates with the amount of PSA and androgen (tightly-regulated suicide gene expression kills PSA-expressing prostate tumor cells).

Another bacterial toxin widely used in suicide gene therapy is streptolysin O (SLO). This oxygen labile hemolytic exotoxin is secreted by streptococcal bacteria and possesses a single polypeptide chain with a molecular weight of 57 kDa (49). This biological molecule, in fact, is a prototype member of pore-forming bacterial cytolysins that can binds specifically to membrane cholesterol, and is oligomerized to create a ring structure in the cell membrane (50). The next consequence is creation of large membrane pores and the increasing membrane permeability to extracellular DNA, RNA, peptides, and proteins; and the final destination of cell is necrosis and death (51). The genes encoding this cytolytic protein can be exploited for cancer gene therapy. There are some *in vitro* studies and clinical trials that applied this strategy. Yang *et al*. showed that transient transfection of the SLO gene could efficiently eradicate cancer

cell lines after a half of day transfection (suicide cancer gene therapy using pore-forming toxin, streptolysin O) (51). In a patented study, the administration of a genetically-modified adenovirus encoding SLO formed pores in the cellular membrane and increased its permeability, which finally killed the transfected tumor cells (52).

Pseudomonas exotoxin (PE) is another protein used in bacterial toxin gene therapy. This 66-kDa cytotoxic proenzyme is secreted by Pseudomonas aeruginosa, in a selective iron-limited media (53, 54). This toxin assists the pathogenic microorganism to invade the human tissues and enter to the cytosol via LDL-receptor (55). The activation of this proenzyme occurred in the cytoplasm when two subunits of protein were cleaved by cytosolic endonucleases. Then, the active C-terminal subunit was attached to eEGF-2 and inhibited protein synthesis, which finally led to cell apoptosis through adenosine diphosphate ribosylation (56, 57). Using a suicide gene therapy approach, Cao *et al*. exploited a truncated version of PE gene to achieve cancer cell targeting and cell death (58). This endotoxin was patented for treating cancer through recombinant nucleic acid constructs (59).

In addition to these common endotoxins, some other bacterial toxins were studied for gene therapy, including: *Clostridium perfringens* enterotoxin (60), bacterial MazF-MazE interferase toxin-antitoxin system (61), and *E. coli* guanine exchange factor (GEF) (62, 63). Moreover, some viral toxins can be added to the list of anticancer genes, such as: the E gene derived from phiX174 (64) and chicken anemia virus-derived Apoptin protein (65).

2.3. Approach (c): introduction of pro-apoptotic genes

Although approach (b), i. e. introduction of genes encoding a cytotoxic protein such as bacterial toxins, could induce cell apoptosis and death, this strategy unfortunately triggered serious undesirable side effects including uncontrolled inflammation because of induction of immune system (66). On the other hand, in the recent years, the mechanism of molecule triggering in physiologic cell death, i. e. apoptosis, was clearly understood. Apoptosis, as mentioned in the previous sections, is a process of mammalian programmed cell death that happens in multicellular organisms in which very important biochemical and morphological changes occurs due to accurately regulated molecular events or signaling cascades (67). The final consequence of this phenomenon is significand cell morphology changes such as blebbing, cell shrinkage, nuclear fragmentation, chromosomal nucleic acid fragmentation, and cytosolic RNA disruption (67). Therefore, a novel approach for the successful treatment of tumor cells is introduction of targeted pro-apoptotic therapeutic genes or development of apoptosis-inducing genes, specially to the cancer cells that suffered a deficiency in apoptotic signaling and thus inadequate apoptosis. This strategy is the straightest approach for cancer therapy in which several machineries and molecules involved in apoptotic signaling or regulation can be targeted. Tumor suppressor genes are normal mammalian genes that contribute to the fidelity of the cell cycle replication process (67). These series of genes negatively regulate oncogenes, decelerate cell division, repair nucleic acid errors, or induced programmed cell death when needed (68). Therefore, the products of these genes protect cells from one step on the path to cancer. Deletions, nonsense mutations, frame-shift mutations, insertions, or missense mutations in tumor suppressor genes, which inactivate or reduce functional activity of their products, lead to the outgrowth of a population of clonally derived tumor cells (69, 70). Strategy (c) is used to offset this disorder. In this approach, the mutant proapoptotic genes were replaced by a complete gene sequence of a tumor suppressor gene that normally induces programmed cell death or cell suicide.

The genes responsible for apoptosis induction, mediation, or execution are routine targets for introduction to tumor cells and for replacing their defect genes. Since one of the most frequent abnormalities identified in mammalian cancer cells is mutant forms of p53 gene, it seems that restoring the proper function of p53 protein may establish the process of apoptosis in these defective cells. Many efforts have been made in this way from years ago. In a successful attempt, "gendicine", the first clinically approved gene therapy

product, have been designed. This engineered adenoviral vector containing wild-type p53 gene can enter the tumor cells through receptor-mediated endocytosis and overexpress genes encoding p53 (71, 72). The next process is programmed cell death (apoptosis) regulated by caspases, some cysteine proteases that play crucial roles in inflammation, apoptosis, pyroptosis, and necroptosis (73).

TRAIL or Apo2L (TNF-related apoptosis-inducing ligand) is also a commonly used apoptosis inducer in tumor therapy. It is well-understood that decoy receptors TRAIL-R3, TRAIL-R4, and TRAIL-R5 is usually expressed on the untransformed cells and TRAIL-R1 and TRAIL-R2 are mainly found on the cell surface of tumor cells (74). Therefore, targeting tumor specific TRAIL could be an interesting way for killing cancer cells without affecting adjacent normal cells. In a study, the possibility of TRAIL gene transfer was evaluated for cancer suicide therapy (75). However, intratumoral administration of TRAIL gene has limitations and their full potential is restricted (76).

There is a recent suicide gene therapy patented system that provides a recombinant gene containing a nucleic acid sequence encoding a cell death mediator protein (CDMP), and activate the apoptotic pathway after expression by tumor cell translation machinery. Interestingly, this system can be inducible by iCaspase-9 and targets a neural cell-specific regulatory element (77).

In addition, there are several pro-apoptotic and apoptotic molecules and cell death mechanisms including Smac, caspase 3, and Bcl-2 that are somewhat considered as anticancer targets for gene therapy (78-80). Among them, apoptosis regulator Bax protein is the most famous protein, which is involved in a wide variety of cellular activities and known as apoptotic activator and central cell death regulator (81). Since a transformed cell carries a truncated form of Bax gene, the apoptosis process is disrupt and the cell becomes resistant to chemotherapeutic agents (82). Therefore, a novel approach for cancer gene therapy is increasing the activity of the Bcl-2 gene family antiapoptotic members or decreasing the Bcl-2 activity.

While suicide gene therapy by pro-apoptotic genes was considered as main strategy, this should be noted that the apoptotic pathways and regulatory mechanisms in both normal and tumor cell are the same. Therefore, it is crucial that at least one strategy must be exploited to limit the molecular targeting only to tumor cells. Without the proper specification, the therapeutic protocol may be failed; just like the failure is shown when and anticancer drug, e.g. the small molecule kinase inhibitor, was applied.

3. Which types of cancers was assessed?

Among the mentioned strategies, suicide gene therapy has been widely validate for treating a variety of cancer types, including colon, liver, lung, medulloblastomas, neuroendocrine, spinal cord tumors, prostate, breast, bladder, brain, gliomas, head and neck, sarcomas, and ovaries (6). Moreover, this therapy has been used as stimulator of cytokines such as interleukin-7 and interleukin-12 as well as anti-angiogenesis agent (83). These in vitro and in vivo experiments and sometimes pre-clinical studies interestingly have proved that suicide gene therapy is more efficient in drug-resistant cancer cells and improve the efficacy of radiotherapy (84). However, only a few clinical trials have been undertaken in humans using the above-mentioned experimental strategies. Some of these trials and their results are briefly cited in Table 3. Given many unknown aspects of suicide gene therapy, it is obvious that there are many concerns about the administration of genetic materials to humans. Therefore, there are limited options for clinical trials associated with suicide gene therapy and most of them are generally highly aggressive and have a poor prognosis.

3.1. Glioblastoma Multiforme (GBM)

GBM, the most prevalent and most malignant brain cancer in adults, is an incurable form of glial tumors (85). It is estimated that more than 90% of patients will die within the first year of diagnosis and the average survival time of newly diagnosed patients is about 15 months (86). Since traditional cancer therapy has not been successful in treating this type of tumor, many efforts have been made to deliver suicidal genes into gliomas. HSV-TKase + ganciclovir therapy is one of the first and most widely performed gene therapy approaches in human trials for gliomas. In a pilot

Table 3. Suicide gene therapy clinical trials.

Type of cancer	Phase of trial	Response	Suicide system used	Author	Year
Metastatic colorectal cancer	Completed Phase I	30% of patients with metastases exhibit response	Intratumoral injection of a replication-deficient adv. CDase gene in presence of 5-FC prodrug	Crystal RG. *et al.*	1997
Brain tumors	Phase I	Limited local antitumor activity in human tumors	Adv. TKase + ganciclovir system	Ram z. *et al.*	1997
Glioblastoma multiform	Phase III	No therapeutic benefit of retrovirus mediated HSV-TK gene therapy	surgical resection and radiotherapy plus adjuvant replication-competent retrovirus mediated HSV-TKase + ganciclovir gene therapy	Rainov NG.	2000
Glioblastoma multiform	phase I/II	Inhomogeneity of tissue formulation distribution	HSV-1-TKase liposomal vector	Voges J. *et al.*	2003
Metastatic Colorectal Adenocarcinoma	Phase I	The therapy was safe and promising efficient with incomplete tumor necrosis	Adv. TKase + ganciclovir system	Sang WM. *et al.*	2001
Small and non-small cell lung cancer	Phase I	Survival and malignant pleural effusion control with a higher efficiency observed for SCLC	Adv.CDase + prodrug 5-FC	Zarogoulidis P. *et al.*	2012
Newly-diagnosed prostate cancer	Phase II/III	Initiated and recruiting at the time of publication	Ad5-yCD +Mutant TKase / 5-FC and valganciclovir prodrug therapy	Lu M. *et al.*	2011
Prostate cancer	Phase I	Transgene expression up to 3 weeks, PSA decline, Acute urinary and gastrointestinal toxicities	CDase + HSV-1 TKase and 3D CRT	Freytag S. O. *et al.*	2002
Breast cancer	Phase I	Efficient selectivity against erb-2	Therapeutic cassette that contains the Escherichia coli cytosine deaminase gene drivan by the tumor-specific erb-2 promoter	Pandha H. S. *et al.*	1999
Hepatocellular carcinoma	Phase I	Recurrence free survival	Adjuvant ADV-TKase	Li N. *et al.*	2007
Prostate cancer	Phase I	No serum cytokine changes after treatment, decreased PSA values, Increased CD8+/HLA-DR+ This study confirmed the safety profile at the surrogate marker of HSV-TKase gene therapy.	Ad.HSV-TKase + ganciclovir	Nasu Y. *et al.*	2007
Head and Neck cancer	Phase I	Local response	Intratumoral RV-HSV-TKase + GCV	Xu F. *et al.*	2009
Esophagus adenocarcinoma refractory cancer	Phase I	Salmonella bacterium can be utilized as a delivery vehicle for the cytosine deaminase gene to malignant tissue with low dose 3 × 107 CFU/m2efficiently.	TAPET-CD	Nemunaitis J. *et al.*	2003
Hepatocellular carcinoma	Phase I	60% of patients demonstrated tumor stabilization of the injected lesion with signs of cell necrosis	HSV-TKase + ganciclovir system	Sangro *et al.*	2010
Pancreas cancer	Phase I	Augments radiotherapy treatment of pancreatic cancer without excessive toxicity	Ad5-yCD/mutTKSR39rep-ADP HSV-1 TKSR39	Freytag S. O. *et al.*	2003
Esophageal cancer	Phase I	TNF erade, in combination with chemoradiotherapy, is active and safe	adenovirus-delivered TNF-α	Chang *et al.*	2012
Intermediate-risk prostate cancer	Phase II	Reduction in positive biopsy results at 2 years in men with intermediate-risk prostate cancer	Ad5-yCD/mutTKSR39rep-ADP in combination with Radiation (IMRT)	Freytag S. O. *et al.*	2014
Recurrent gynecologic cancer	Phase I	Five patients showed a stable disease; all others experienced progressive diseases	Ad5.SSTR/TK.RGD/Ganciclovir (GCV)	Kim*et al.*	2012

clinical trial followed by a phase I/II study, 8, 12, and 30 patients with recurrent glioblastoma multiforme received intratumor injection of combined IL-2/HSV-TKase gene, followed by a systemic administration of ganciclovir (87). The results of this clinical trial showed that the therapy was tolerated without major side effects, effectively transduced the combined suicide genes to glial cells, and activated a systemic cytokine cascade, with a tumor response in 50% of cases (88). Some other finished or in-progress trials using this system but with different delivery systems demonstrated nearly the same results and anticipated a bright future for gene therapy of GBM (89, 90).

3.2. Breast Cancer

The second leading cause of cancer-related death among women is breast cancer (91). In recent years, several new biologic and chemotherapeutic agents have been developed for patients with breast cancer. However, the life expectancy for patients with metastatic form of breast cancer is still limited (92). Therefore, some strategies based on suicide gene therapy systems were developed. The first cancer clinical trial using CDase/5-FC system was done in human subjects suffering breast cancer by pandha et al. in 1999, which specifically controlled expression of erbB-2 oncogene in erbB-2-positive tumor cells by overexpression of the suicide gene (93). The approach was considered as a safe method that encouraged the development of genetic prodrug activation therapies (94). Since this study proved promising results for treatment of cancer cells with the CDase/5-FC system, a number of other clinical trials using this system was also conducted towards different types of cancers. Brade *et. al*. also designed a clinical trial for breast cancer patients using heat-directed suicide gene therapy delivered by an adenoviral vector (95). They used a dual prodrug-activating *E. coli* CDase/HSV-TKase fusion gene under the control of the hsp70b promoter followed by administration 5-FC and ganciclovir with a mild hyperthermia. The authors stated that this combined suicide gene therapy was highly effective against heat- and radiation-resistant breast cancers.

In addition to breast cancer, suicide gene therapy was applied in some women with other sex-related cancers. The effectiveness of HSV-TKase suicide gene in woman with gynecologic cancer (endometrial and ovarian) have also been studied by using in human trials. In a phase I clinical trial, HSV-TK gene was successfully transfected to patients with recurrent gynecologic cancer using an infectivity-enhanced adenovirus and then the prodrug ganciclovir was administrated (96). This study revealed that this strategy is safe and efficient for patients with recurrent gynecologic cancer with a capacity of technical imaging.

3.3. Prostate Cancer

The usual treatment regimen for prostate cancer is external beam radiation therapy. Despite prolonging survival, it is only valid for non-metastatic cancers (97). In addition, long-term radiotherapy is associated with complications that are sometimes not tolerated by the patient and are very genotoxic (98). Therefore, looking for a pharmacologic or biologic way for treating prostate cancer or finding a better approach to improve the efficacy of radiotherapy seems more avantageous. In fact, it's much better to find a targeted therapeutic substance, rather than increasing the prescription radiation dose. It seems that the concept of using suicide gene therapy may be considered as a great alternative or combination approach to achieve this goal. Freytag *et al.* performed a phase I trial in which a replication-competent adenovirus delivered CDase/HSV-TKase fusion gene to tumors followed by administrating 5-FC and ganciclovir prodrug (99). This phase I study was the first gene therapy trial in patients who were presented with local recurrence of prostate cancer. This study utilized a replication-competent virus and were done into 16 patients. 44% of cases revealed a greater than 25% decrease in serum prostate-specific antigen (PSA) as the tumor marker, and 19% showed a greater than 50% decrease in PSA. The results showed that this regimen was a safe therapy applied to humans and the therapeutic agents were biologically active. The authors are continuing this method and the results of their second publication verified their previous theory. In the second clinical trial, this team used this therapeutic suicide gene therapy system for newly diagnosed, intermediate- to high-risk prostatic cancer patients

(100). This time, they combined the suicide gene with conventional-dose three-dimensional conformal radiation therapy for 15 patients. The results again confirmed that this therapy was safe and PSA was significantly decreased in all patients. Another team conducted a phase I study of in situ HSV-TKase + ganciclovir (GCV) system for eight patients with hormone-refractory prostate cancer. In addition to safety profile, this trial emphasized the possibility of clinical response at the surrogate marker level (101).

A different suicide gene therapy system, using bacterial nitroreductase (NTR) gene was also applied for treatment of localized prostate cancer. In this phase I/II clinical trial, some patients were intraprostatically injected Ad/NTR in combination with prodrug patent code CB1954 (102). The results revealed that this therapy was well tolerated and progression of PSA in treated patients was delayed.

3.4. Liver cancers

Hepatocellular carcinoma, the third leading cause of cancer-related death, was also subjected to suicide gene therapy. In a phase I clinical trial, Sangro *et al*. intratumorally administrated a HSV-TKase + ganciclovir system to patients with advanced hepatocellular carcinoma (103). They showed that this regimen was well tolerated and any significant toxicity, even hepatic toxicity in cirrhotic patients, was not observed. In the presence of common minor side effects, such as flu-like syndrome and fever, 60% of patients demonstrated tumor stabilization of the injected lesion, and sometimes signs of intratumoral necrosis was observed.

In addition to the mentioned types of cancer, suicide gene therapy was evaluated for the treatment of some other complicated and metastatic cancers. In a phase I clinical trial performed by Nemunaitis *et al*. TAPET-CD, a bacterial CDase gene therapy system, was used in refractory squamous cell/head and neck and esophagus adenocarcinoma cancer patients (104). They injected this attenuated bacterial vector into the complicated tumor cells of three patients. It was reported that this regimen had minimal adverse effects and the delivered gene was found to be functional in converting 5-FC to 5-FU in the malignant tissue. Therefore, it was the proof of concept of bacterial vectors for gene therapy as a notable system and showed that the delivered genes were able to convert the intended prodrug in targeted.

4. Conclusion

Conventional cancer therapy including chemotherapeutic agents and tumor surgeries is limited due to treatment insufficiency besides many unbearable adverse effects. In recent century, a breakthrough have been raised in the development of gene delivery and expression systems, and scientist hope to use these systems in formulation to clinical demands. Suicide gene therapy strategies represent new promising approaches to get highly specific cancer therapies in which high-toxic antineoplastic drugs can be delivered directly to cancer cells, thereby minimizing toxic effects. Several suicide gene therapy systems have been designed according to the three major strategies, including: introducing prodrug activating suicide gene or delivery of bacterial toxin and apoptotic genes. On this basis, several delivery and effector systems were developed for suicide gene therapy, which showed effectiveness against many types of cancer cells, albeit often in vitro and in pre-clinical studies. Nevertheless, we are just in the beginning of a long way to realize these new treatments offer and the clinical trial in humans is still limited by the low potency of gene delivery vectors and also safety and ethical issues. Therefore, the clinical trials on some cancer types were performed in limited numbers and the results were modest. However, novel pathways such as targeted suicide gene therapy and new powerful vectors and promoters as well as seeking for an efficient combination therapy are under intense investigations.

Conflict of Interest

None declared.

5. References

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