The Suicide Gene Diphtheria Toxin A Based Therapy in Cancer Treatment

Anh Nguyen.T.Q[1] and Dong Kee Jeong[1,2,†]

[1]*Department of Animal Biotechnology, Faculty of Biotechnology, Jeju National University, Jeju 690-756, Korea*
[2]*Sustainable Agriculture Research Institute (SARI), Jeju National University, Jeju 690-756, Korea*

ABSTRACT : Therapeutic cancer is a long lasting and turbulent history accompany with the milestones in surgical intervention, chemotherapy and radiotherapy. In the past decade, however, metastatic cancer still obstinately exists challenging the professional scientist. Beside the major forms of cancer treatment, Diphtheria toxin (DT) which is produced by a pathogenic strain of bacterium *Corynebacterium diphtheria* to shield themselves against the other dangerous organism, have been researched as a potential candidate to overcome the drawback such as non-specific, non-effect to drug resistant cancer cell and side effects when using chemotherapy and radiotherapy. In the context of suicide gene therapy, the DT expression under controlling of tissue-specific promoter will be targeted in cancer cell but defect in normal cell. The molecular mechanism, characteristic of DT-bases therapy and prominent achievements of preclinical and clinical studies for the past decade are summarized and discussed in this review.

Key words : Diphtheria toxin A, Suicide gene, Tissue-specific promoter, Gene delivery

INTRODUCTION

Therapeutic cancer is a long lasting and turbulent history accompany with the milestones in surgical intervention, chemotherapy and radiotherapy. However, metastatic cancer still obstinately exists challenging the professional scientist. In this case, gene therapy emerged as replacing candidate with full of honor including the delivery of anti-angiogenic factors, tumor-suppressor or apoptotic genes, prodrug-activating genes and immune-stimulating genes. According to the statistics of the Journal of gene medicine database, 63.4% of total clinical gene therapy trials have concerning with cancer in 2003 and continuously increase in recently. Among these approachs in apoptotic gene delivery, DT which is produced and released extracellularly by a pathogenic strain of bacterium *Corynebacterium diphtheria* was received the attentions more or less (Collier, 1975). This exotoxin inhibits a growth of eukaryotic cells by stopping protein synthesis via catalyzing adenosine diphosphate (ADP) ribosylation of the diphthamide group of cellular elongation factor 2 and kills cells through an apoptosis pathway such as defense mechanism itself with other living creatures around. An effect of diphtheria toxin was firstly described as a protein synthesis inhibitor in the metabolism of Hella cell (Strauss & Hendee, 1959).

Over several decades, based on cytotoxic effect, DT have been researched as a potential candidate which could be overcome the drawback such as non-specific, non-effect to drug resistant cancer cell, side effects when using the chemotherapy, the radiotherapy in cancer treatment. Two major strategies which take advantage from understanding molecular mechanisms of cancer cell are immunotoxin targeted therapy and suicide gene therapy (Robert, 2006; Shapira, 2010). In immunotoxin targeted therapy, A fragment of Diphtheria toxin (DT-A) take a role as toxin moiety while B-fragment diphtheria toxin (DT-B), which was genetically manipulated or not, refer to target moiety that was known as ligand or antibody bind to specific receptor on surface cancer cell. The suicide gene therapy focus on expression of DT-A under controlling of intercellular

† Corresponding author: Dong Kee Jeong, Dept. of Animal Biotechnology, Faculty of Biotechnology, Jeju National University, Jeju 690-756, Korea. Tel: +82-64-754-3331, Fax: +82-64-725-2403, E-mail: dkjeong@jejunu.ac.kr

transcription factors which is abundant in cancer cell but defect in normal cell. This review will supply the particular traits of Diphtheria -based gene therapy approaches in cancer treatment and summary the prominent achievements which have been evaluated via preclinical and clinical studies of each approach. In addition, obstacles in treatment and strategies to rebound these limitations also were considered.

STRUCTURE AND MECHANISM OF DIPHTHERIA TOXIN

DT is single polypeptides chain (62,000 Daltons) of 535 amino acids including two functionally distinct fragments: A fragment (21,150 Daltons) is responsible enzymatic activity and B fragment (40,700 Daltons) represent to a translocation receptor which binding to cell surface receptor. The three-dimensional structure of DT shows that it actually consist of 3 secluded domains: the N terminal domain which is A fragment represents the catalytic properties (C-domain, 1-188 residues), the C terminal domain represents the B fragment is divided into two regions: receptor binding domain that binds to specific cell receptor (R domain, 387-535 residues) and the translocation domain which join the interaction of fragment B with the cell membrane bilayer (T domain, 200-387 residues) (Choe et al., 1992; Lambotte et al., 1980).

The heparin-binding epidermal growth factor precursor is known as natural receptor of the native diphtheria toxin (Louie et al., 1997). The DT sensitivity of cells is depended on the presence or absence of that DT receptor. DT bind to epidermal growth factor (EGF) like domain of human membrane-anchored heparin-binding EGF like growth factor (proHB-EGF) but does not bind to mouse proHB-EGF because of amino acid substitution in the EGF-like domain (Honjo et al., 1971). There are many studies demonstrates that some amino acid residues in the region of 122-148 residues of EGF like domain as Glu 141, Phe 115, Leu 127, IIe-133 and His-135 amino acid take a critical for DT binding activity (Mitamura et al., 1997; Jeong et al., 1998). Once binding to receptor, A fragment is internalized into cytosol and ADP-ribosylase of elongation factor 2 (eEF2) which lead to translation inhibition. In detail, DT undergoes these steps to cause death of cell: (1) DT bind to the cell surface receptor via its R domain (residues 482 -535) (Rolf, 1990) (2) DT internalized into clathrin coated pits and go to endosome, subsequently DT unfold because of acidic environment inside endosome (Paul et al., 1996; Lemichez et al., 1997). The disulfide bond between Cys 186 and Cys 201 that link translocation domain (T) to catalytic domain (C) is reduced by cell furin protease (Chiron et al., 1994; Tsuneoka et al., 1993). (3) A hydrophobic region of the T domain changes to hairpin form and insert into the membrane of endosome, forming the channel through which proteolysis fragment-C domain translocate and move to cytosol. (5) In the intercellular, the active side cleft of catalytic fragment (amino acids 34-52) bind to nicotinamide adenine dinucleotide (NAD^+) and the ADP-ribose group of NAD^+ is transferred to eEF2 that lead to inactivates translation of eEF2 (Morimoto et al., 1992; Wilson et al., 1994; Deng et al., 2008). (6) Cell death is facilitated by inhibition of protein synthesis and apoptosis (Thorburm et al., 2004). The A fragment is acknowledged as an extremely toxic which sufficiently kill one cell with one single molecule (Yamaizumi et al., 1978).

DIPHTHERIA TOXIN BASED SUICIDE GENE THERAPY

The suicide gene therapy bases on the delivery to the targeted cells of genes that are either directly toxic, pro-apoptotic or that have the ability to convert nontoxic pro-drug into active drug. For example, herpes simplex virus thymidine kinase (HSV-tk), cytosine deaminase and purine nucleoside phosphorylase is the prominent drugs activating enzyme which not only kill a transducted cell but also any neighboring non-expressing cell because of "bystander effects". While mean, DT-A released from the

lysed cells is not able to damage the contiguous cells in the absence of the DT-B fragment, therefore only specifically kill targeted cells. DT- A expression has shown that the toxin is so potent the cell death occurs before enough DT-A can accumulate for immunological detection (Li et al., 2002). Another advantage of DT-A is that in contrast to other suicide genes that act by disrupting deoxyribonucleic acid (DNA) synthesis, thereby targeting rapidly dividing cells, DT-A is a potent inhibitor of protein synthesis that acts in a cell cycle-independent fashion, killing both quiescent and rapidly dividing tumor cell (Rodriguez et al.,1998). This property of DT-A suggest that DT-A therapy may be especially suited for slow growing tumor like prostate tumors or aggressively growing tumor like melanoma and variety of different kind of cancer. As mentioned above, that the minimal amount of DT-A is extremely toxic enough to kill a cell. Therefore, to avoiding any unintended deleterious effects on normal cell, the two challenges which the DT-A based therapy must be overcome is high transducted efficiency and the expression of gene in specific target cells. Several attempts in the limiting toxicity of DT-A such as using a modified metallothionein promoter (Maxwell et al., 1986), prokaryotic control element (Robinson & Maxwell, 1995) or replacing wild-type DTA sequence with attenuated mutant of DTA (Maxwell et al., 1987) sequence were still unable to achieve complete eradication of undesirable cell killing. Identification of tissue and tumor-specific promoter elements for targeting expression of therapeutic and toxic genes to tumor cell is critically important for the practical application of gene therapy (Shirakawa et al., 1998; Lee et al., 2002). Ideally, promoter-element-should-direct expression of the therapeutic DNA to tumor cells, and these should be no expression of the therapeutic DNA in normal, noncancerous cells. The many of tissue-specific promoter for each of cancers have been detected and applied for the controlling of DT-A's expression for over decades. Most of them such as prostate-specific antigen (PSA) promoter, human chorionic gonadotropin (hCG), human carcinoembryonic antigen (CEA), hepatocellular carcinoma (HCC) were evaluated at preclinical phase. In parallel, the new adjunct portions in non-viral and viral vector systems which improve transfer-gene efficacy as well as tightly regular targeted gene also have been applied.

TISSUE-SPECIFIC PROMOTER IN DTA BASED SUICIDE GENE THERAPY

The targeted diseases of DT-A suicide therapy was variety of cancer namely prostate cancer, ovarian cancer, bladder cancer, pancreatic cancer, glioma, B-Lymphoid cells, melanoma. Base on the discovery of the sequence of tissue and cancer-specific gene regulation in organogenesis and cancer fields has facilitated the development of new promoter system to express therapeutic gene DT-A only in targeted cell and tissue. Many of them were expressed in a wide spread in

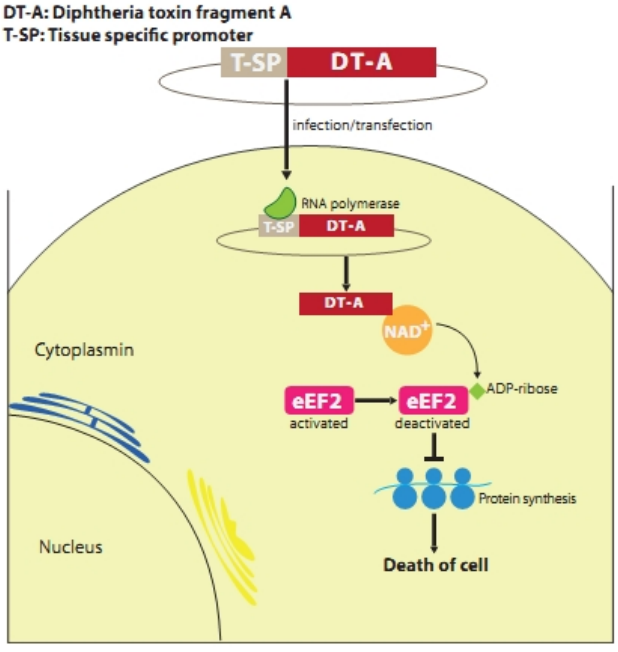

Fig. 1. Mechanism of the suicide gene DTA expression under the tissue specific promoter controlling inhibit protein synthesis in intercellular. Abbreviation: NAD^+ -nicotinamide adenine dinucleotide; DT-A - Diphtheria toxin A; eEF2 - elongation factor 2; T-SP: tissue-specific promoter; ADP-ribose-adenosine diphosphate ribose.

Table 1. Summary of tissues/cancer specific promoter used in preclinical evaluation of the suicide gene diphtheria toxin A based therapy

Cancer disease	Tissues specific promoter	Delivery vectors	Referrence
Protaste cancer	PSA	Adenovirus	Peng et al., 2002 & 2005
		Lentivirus	Zheng et al., 2003
		C32 - Cationic polymer	Peng et al., 2007
		C32-117 cationic polymer	Anderson et al., 2005
Ovarian cancer	H19	Cationic polymer	Mirzahi et al., 2009 & 2010
	hCG	Electroporation	Lidor et al., 1997
	HE4, MSLN	Cationic polymer	Huang et al., 2009
Bladder cancer	H19	Calcium phosphate	Smaldone et al., 2010
	H19/IGF-P4	Cationic polymer	Ohana et al., 2002
			Amit & Hochberg, 2010
	hTER,hTERT		Abdul et al., 2000
	IGF2/P4, IGF2/P3	Cationic polymer	Ayesh et al., 2003
			Amit et al., 2011
Glioma cancer	GFAP	Baculovirus	Wang et al., 2006
	MBP	Retrovirus	Martin et al., 2000
Pancreatic cancer	MSLN		Showalter et al., 2008
	Hsp70B'+HSEs	Cationic liposome	Fogar et al., 2010
Pituitary tumor	GH	Adenovirus	Lee & Jameson, 2002
Colorectal carcinoma	CEA	Cationic liposome	Cao et al., 1998
Melanoma	MIA, tyrosinase	Cationic lipid	Rothfels et al., 2003
Hepatocellular carcinoma	AFP	Cationic liposome	Murayama et al., 1999
			Kunitomi et al., 2000
B-lymphoid cell	Immunoglobulin heavy/ κ -light chain	Electroporation	Maxwell et al., 1991

PSA- prostate-specific antigen; hCG - human chorionic gonadotropin; HE4-human epididymis protein 4; MSLN-mesothelin; IGF-P4 - insulin like growth factor- promoter 4; hTER, hTERT -Transcriptional Regulatory Sequences of Telomerase; GH -growth hormone; CEA - human carcinoembryonic antigen; AFP - alpha-fetoprotein; MBP - Myelin basic protein; GFAP - glial fibrillary acidic protein

various cancers, so the design of a universal promoter to targeting many different cancers is possible. Prostate cancer, ovarian cancer, bladder cancer couple with their specific promoter will be introduced in detail following.

1. Prostate Cancer

Prostate cancer a form of cancer that develops in a gland in the male reproductive system is the most commonly diagnosed malignancy and the second leading cause of cancer death of men in the United States (Shannon et al., 2001). Prostate cancer is type of slowly growth and aggressive cancer which metastatic to the other organ of the body, especially in bones and lymph nodes. This makes prostate cancer is an attractive candidate for the DT-A based therapy. In addition to, prostate-specific antigen (PSA) a member of the kallikrein family of serine proteases (Clements, 1989) involved in prostate growth regulation by cleaving insulin-like growth and thereby increasing the bio-availability of insulin-like growth factors (Doherty et al., 1999; Sutkowski, 1999). PSA gene regulatory regions are attractive candidates to control prostate-specific expression.

To tightly regular the expression of DT-A in prostate cancer cell, site directed recombinase enzyme were firstly described (Peng et al., 2002*)*. This strategy relied on both transcriptional regulation and regulated DNA recombinase mediated by a site-direct DNA recombinase enzyme, flipase (Flp) recombinase. In those cells, the expression of the recombinase which was controlled by cell-specific promoter

will lead to recombination of targeting sequences. Following intratumoral injection of adenoviruse of DNA encoding enhancer/promoter sequence of the human prostate-specific antigen (PSA) gen, PSE-BC promoter-driven Flp recombinase and DT-A, they demonstrate that this dual system control strategy effectively activates DT-A expression in a manner that correlates with the amount of PSA and androgen in cell. The size of xenograft was reduced and apoptotic cell in prostate tumor in the transgenic adenocarcinoma of the mouse prostate (TRAMP) mice was observed in another study (Zheng et al., 2003). Apoptosis in 80% of tumor cell also observed in C32/DT-A injected prostate tumors of TRAMP mice (Peng et al., 2007).

2. Ovarian Cancer

Ovarian cancer is one of cancer occurring in genital system, and a fourth cause of death from cancer in women in western countries. In 2011, over 21.990 new cancer case and 15.460 death case in both of gender were reported in United States (Siegel et al., 2011). An advantage-stage epithelial ovarian cancer is frequently treated by optimal surgical debulking followed by chemotherapy and about 80% of these case, recurrence were acknowledge, unfortunately (Dinh et al., 1992). The alternative therapy is need immediate in order to improve rate of fatal. Recently, H19 regulator sequences emerged as potential candidate for controlling deliveried-DNA expression in various type of cancer. The H19 gene is an imprinted, maternally expressed gene in humans that tightly linked and co-regulated with the imprinted, paternally expressed gene of insulin-like growth factor 2(IGF-2) (Pachnis et al., 1988; Rachmilewitz et al., 1992). No protein product is translated from H19 mRNA and their function still no clearly. This RNA is highly abundant during embryogenesis in mesodermal and endodermal tissues and not expressed in normal adult tissues (Poirier et al., 1991; Lustig-Yariv et al., 1997). Interesting, H19 RNA is detected in many of human cancers such as bladder carcinoma (Cooper et al., 1996), lung cancer (Kondo et al., 1995), breast adenocarcinoma (Dugimont et al., 1995), cervical carcinoma and esophageal cancer. Patricia Ohara et al. have reported the bladder cancer cell killing activity by the DT-A expression driven by H19 regulatory sequences in an animal model of bladder cancer induced by subcutaneous injection of bladder tumor cell line (Ohana et al., 2002). Aya Mizrahi et al. (2009) determined the hight level of H19 RNA found in 90% patients with epithelial ovarian cancer in their experiment and intratumoral injection of DTA-H19 into subcutaneous tumors induced in mice caused at least 40% inhibition of tumor growth . Moreover, introduction of H19 regulatory sequence into cancer cell do not trigger expression of endogenous H19 which might stimulate cancer process. With plentiful expression in many kind of cancer cell as mention above, DTA-H19 become a "multi-potent vector" and have been assessed in pre-clinical and clinical level by scientist in world. Nowadays, DTA-H19 was named commercial BC-819 in development by BioCancell Therapeutics Inc. Most of *in vivo* studies concentrated in the treatment of bladder cancer of BC-819 with the delivery strategy is that DTA-H19 is mixed with a transfection agent for bladder instillation. A phase I/IIa clinical trial in patients with non-muscle invasive bladder cancer receiving intravesial BC-819 reported mild local toxicity and complete and partial response rates of 22 and 44% (Smaldone et al., 2010). Other clinical trials with colon, ovarian and pancreatic cancer also yielded impressive results.

3. Bladder Cancer

Bladder cancer is the common cancer of urinary system with a high incidence; approximately 69,250 estimated newly cases and 14,990 deaths in the United States in 2011. Although the high success rate in treatment by surgical transurethal resection (TUR) of tumors, the recurrent cancer was seen in 70% of patients (SchenK-Braat & Bangma, 2005). Therefore, DT-A based therapy were also applied to pass the obstacle associated with chemotherapy and immunotherapy in cancer therapeutics.

As above, IGF-2 is a paternally imprinted gene in human

and takes important role in embryonic growth. With different 4 promoters, IGF-2 is transcribed in embryonic stage and adult cell by P3/P4 promoter and P1/P2 promoter, respectively (Pagter et al., 1987; 1988; Holthuizen et al., 1990; Engstrom et al., 1998). However, the over-expression of IGF-2 under P3/P4 promoter activation were detected in tumor development such as bladder carcinoma, hepatocellular, breast cancer and prostate cancer make this IGF-2 regulator sequences become an attractive candidates for target cell killing by DT-A (Sohda et al., 1996; Bae et al.,1999; Hahn et al., 2000; Lee et al., 2000; Mineo et al., 2000). Ayesh et al used IGF-2 promoter sequences to regulate specific DT-A expression in human bladder carcinoma cell lines after detecting hight level of mRNA expression from P3, P4 or both promoters in 62% of transitional cell carcinoma samples. toxin activity of both DT-A/P3 and DT-A/P4 expressing vectors were determined in cell line *in vitro* and at least 70% inhibition in tumors intra-tumoraly administration with DT-A/P3 compare to non-injected tumors were observed. Later, the double promoter P4-DTA-P3-DTA vector was designed with aim at eradicating cell which do not express either IGF2-P3 or IGF2-P4. In heterotopic bladder tumors, this double promoter P4-DTA-P3-DTA vectors displayed predominant inhibition compared to the single promoter vector and the average size of tumors was 83% smaller than that of the control group (Amit et al., 2011).

However, as not all cancer cell expressed high level of IGF-2/P4 example as IGF-2 and H-19 expressed just in 50% and 70% of human bladder carcinomas, respectively (Ariel et al., 2000; Ayesh et al., 2003). Hence, some of patients will not match this this target medicine approach. This reason make Doron Amit et al created a double promoter DT-A expressing vector which have two separate DT-A sequence under the regulatory of H-19 and IGF-2/P4 promoter, respectively (Amit et al., 2010). At the first stage, the total expression of both IGF2-P4 and H19 transcripts was detected at high expression level in 100% (29/29) of the trasition cell carcinoma (TCC) samples by the quantitative real time polymerase chain reaction (qRT-PCR) and *in situ* hybridization (ISH) method. After that, double promoter contructs H19-DTA-P4-H19 was evaluated in TTC cell line *in vitro* and show extreme inhibition compared to each of the single contructs H19/DTA or P4/DTA. The *in vivo* results in heterotopic and orthotopic of animal model of bladder cancer also support this strategy. In addition, death cell not only take part in destroy of the tumor but also reduce the source of mitogenic IGF-2 which give a hand in the development of human malignancies and metastases process (Kawamoto et al., 1999; Pavelic et al., 2002).

THE DELIVERY OF DT-A CONSTRUCT IN TUMOR CELL *IN VITRO* AND *IN VIVO*

The delivery of therapeutic gene must responded two requirements: efficacy and safety. It's has been shown that the gene dissemination within tumor is significantly limited by quick proliferation, high density, loss of contact inhibition of the cell. Especially with DT-A gene therapy, the gene transfer efficiency is the most important concern because DT-A cannot enter surrounding cell to kill them without DT-B. The various strategies of the gene delivery such as the new revolutions in structure of viral vectors or the new cationic materials for transfect have been applied thoroughly in reclinal.

Ying Li et al. (2002) demonstrate the first successful generation of a DT-A-expressing recombinant adenovirus under the control of PSA promoter. *In vitro* preferential PSA-positive prostate cancer cells killing were shown. *In vivo* the nu/nu mice with PSA-positive cancer cell LNCaP xeno-graft treated with DT-A virus had a rapid regression of tumors and survived over a year without tumor progression. However, the limitations of gene therapy using adenoviral vector is the non-specific expression of therapeutic genes in normal cell causing toxicity to noncancerous tissues, tissue specificity is decreased to approximately 20-fold or even lower with adenoviral vector thus for test

(Latham et al., 2000; Shi et al., 2002). Addition to, adenovirus vector are the most immunogenic of all the viral vector groups, and the largest hurle that has faced gene therapies using adenovirus vectors is overcoming this immunogenicity (Thomas et al., 2004). Regression of prostate cancer xenografts by a lentiviral vector specifically expressing diphtheria toxin A under the controlling of PSA promoter and the upstream regulatory sequence of the PSA gene was reported by Zheng et al, (2003). Approximately 75% of LNCaP prostate xenografts in nude mice were completely eradicated with single injection of the DT-A lentiviral vector. Although less imflammatory and immunogenic than adenovirus, two major disadvantage of using lentiviral vectors is low titer of virus stock, integration of viral genome and component derived from human immune-deficiency virus 1 (HIV-1) make it to be considered seriously in clinical trials.

Chao Yang Wang et al developed a recombinant baculovirus *Autographa california multiple nucleopolyhedrovirus* (AcMNPV) accommodating the DT-A and the promoter of glial fibrillary acidic protein (GFAP) to minimize possible side effects caused by overexpression of a therapeutic gene in sensitive neuron. They demonstrate that using baculovirus AcMNPV as a cancer gene therapy vector not only easy construction of recombinant viral vector but also significantly improved transduction in glioma cell, providing the efficiency in C6 rat glioma cells up to 96%. When used to produce the A-chain of DT toxin intracellularly in a rat C6 glioma xenograft model, the baculovirus effectively suppressed growth of tumor cells in the brain by just one injection of DT-A expressing baculovirus construct (Wang et al., 2006).

As mention above, DT-A toxin fragment cannot penetrate into surrounding cell without help of DT-B fragment. Actually, DT-A could also escape from dead cell and could be taken up by nearby cell through non-receptor-mediated endocytosis. Moreover, fraction of viral virus could leak to capillary vessels and may infect endothelial cells in blood vessels and other organ or tissues as liver, spleen, stomach, lung… Fortunately, histo-pathological examination of internal tissues in many studies didn't show any evidence for toxicity that indicate under the controlling of tissues-specific promoter, intratumoral injection of virus ensure safety for other tissues (Zheng et al., 2003). The "Achilles heel" of gene therapy is that many of the immunological defense systems that are used to tackle wild-type infections are activated against the vectors and/or new transgene products that might be recognized as foreign. In order to overcome this issue several stragies have been examined. Immunosuppressive agents including cyclosporin, tacrolimus (FK506) or anti-CD4 monoclonal antibody have been found to enhance transgene expression of adenoviral vector (Smith et al., 1996; Ilan et al., 1997; Ye et al., 2004). The sequences modifications of viral envelop and capsids have been focused.

To avoid the limitations and serious side effects associated with viral vector, as well as toxicity and poor delivery efficiency associated with nonviral systems, the cationic poly (beta-aminoesters) polimer- C32 are attempted to deliver DNA construct encoding the DT-A gene (Anderson et al., 2004). Under the controlling both by the use of PSE-BC and by DNA recombination mediated by Flp, C32 delivery of DT-A inhibit LNCaP xenograf tumor growth, 40% of treated tumors regress in size. The delivery of nanoparticles of complexion DT-A with C32-117, a modified version of poly (beta-amino ester) C32 (Anderson et al., 2005) the into peritoneal cavity under the controlling of Human epididymis protein 4 (HE4) and mesothelin (MSLN) promoter allow direct treatment of metastatic ovarian tumor were described (Huang et al., 2009). HE4, a marker for ovarian carcinoma, is the product of the WFDC2 gene that is overexpressed in patients with ovarian carcinoma while MSLN protein is overexpressed in epithelial mesotheliomas, ovarian cancers and in specific squamous cell carcinomas (Bingle et al., 2002; Hellstrom et al., 2003; Rosen et al., 2005; Breidenbach et al., 2005; Hellstrom et al., 2008). Expression of nanoparticle-delivered DT-A DNA suppressed the growth of xenograft tumor in immunosuppressed mice as well as primary ascites cells collect from patients more

efficient than in mice treated with cisplatin and paclitaxel. In the further explore, the complex of C32 -PSA/DT-A were injected directly into the right lobe of the normal prostate and to prostate tumors in mice.In the prostate of K5/CFP+PSA/EGFP double transgenic mouse, Cyan Fluorescent protein (CFP) is targeted to be expressed in basal cell in the epithelium of the prostate, Green Fluoresce Protein was targeted to be expressed in luminal cell in the prostate epithelium. After injected, the shutdown of protein synthesis in luminal cell was showed by the decrease of GFP expression. In contract to, GFP expression was similar in both of group which non-injected mice or injected PSA-firefly luciferase (PSA-Fluc) mice. CFP expression in lobes injected with PSA/DT-A or PSA/Fluc nanoparticle was not different (Peng et al., 2007).

The analyses of histologic from non-tumorous tissues and blood chemistry toxicity showed no significant non-specific toxicity except for very low expression was observed in uterus, abdominal fat and intestine. Because of inhibiting of protein synthesis immediately following uptake DT-A, cell will not reveal any symptom of resistance to the nanotherapy extending period of time DT-A administer to suppress tumor growth, and perhaps even reduce tumor burden is possible. This reason make the delivery methods which based non-virus such as cationic polymer, electro-poration, $CaPO_4$ take a majority in this field.

CONCLUSION

Under the control of tissues-specific promoter, the DT-A gene encoding a highly toxic bacterium protein when introduced into the cytoplasm of eukaryotic cells has been investigated in cancer therapy. This gene inhibits protein synthesis by deactivating cellular elongation factor 2 and induces cell death through an apoptosis pathway. Without DT- fragment B, DT- fragment A cannot escape from the cell and invade a surrounding other cells, implying that the sacrificed of undesirable cells is negligible in cancer treatment. By blockading protein synthesis, DTA kill not only quiescent but also rapidly dividing tumor cells that reason DTA therapy adapted to the cure of many kind of cancer. Following, a number of tissue-specific promoters which drive exactly the expression of DT-A gene in cancer cell but not in normal cell namely PSA promoter, IGF-P3/IGF-P4 promoter, H-19 promoter etc. were found out and attempted in pre-clinical experiments. The revolutions in both of structure of vector system and transfer gene technique have contributed in high cure efficiency of the suicide gene DTA based-therapy.

As with the other available toxicity drugs, maximal dose of viral vector which sufficient to treat metastatic cancer *in vivo* if high dose of the DT-A vector do not lead considerable pathogen effects is need to be define. Appropriate animal model is required to investigate the immune-mediated and inflammatory responses and other toxic side effects. The utilizing of viral-mediated gene therapy has been tightly considered in the term of safety in clinical trials. But in field of cancer treatment, this considering may create less of obstacles because the duration of viral gene expression is relatively short. Addition to, viral vector dissemination within a tumor is significantly hindered by poor mobility of viral particles in the tumor mass, and this has been recognized as being one of the major obstacles to achieving good clinical benefits with this form of cancer treatment. Thus, in contrast to the field of gene therapy for non-tumor disease, where safety has been a major concern, for virus mediated tumor treatment, clinical efficacy is the most upfront obstacle at the present time (Jia & Zhou, 2005). In reality, no vector, viral or non-viral is likely to transduce 100% of tumor cells. That reason we propose the suicide gene DTA based-therapy may be applied in combination with other cancer therapy methods, such as surgery, chemotherapy and radiology to achieve high effects in cancer treatment.

In conclusion, the suicide gene DTA based-therapy have been showed attractive approach in cancer treatment research. However, clinical data in this field is scarce and still a long step to go before we can apply knowledge

acquired in the laboratory to the clinical treatment of patients. More extensive research is necessary to improve the efficiency, the ultimate dose of vector and detail strategies in the combination with other cancer therapies.

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