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Review Article

Adenoviral based Gene Therapy for Cancer in Human and Animals: A Review

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ABSTRACT

Adenovirus vector is the most common used vector in clinical gene therapy. The development of adenovirus from the first generation until the helper-dependent adenovirus vector has greatly reduced toxicity and immunogenicity. The helper-dependent adenovirus can also prolong transgene expression. Tissue- or disease-specific approach has been used to improve the specificity of adenoviral vector for cancer gene therapy. This review summarizes some adenoviral gene therapy and targeting approaches available for human cancer as well as animal cancer.

Keywords: Adenovirus vector, clinical, gene therapy, human cancer, animal cancer

ABBREVIATIONS

Adenovirus (Ad)
Alternative reading frame (ARF)
Arg-Gly-Asp (RGD)
Carcinoembryonic antigen (CEA)
Complementary DNA (cDNA)
Coxsackie adenovirus receptor (CAR)
Cytomegalovirus (CMV)
Human papillomavirus (HPV)
Interferon (IFN)
Interleukin 2 (IL-2)
Interleukin 12 (IL-12)
Non-small-cell lung cancer (NSCLC)
Tumour necrosis factor (TNF)
Tumour protein 53 (p53)

INTRODUCTION

Adenoviruses (Ad) are among the most commonly used vectors for the delivery of genetic material into human and animal cells, and in selected stable germ-line (Stephan and Kelly, 2002; Takehashi *et al.*, 2006). In addition, adenoviruses have a number of advantages as vectors; these include a rapid infection of both dividing and non-dividing cells, DNA stability, ease of manipulation and propagation, and low pathogenicity in humans. Adenoviruses became the most widely used vector for gene therapy clinical trials in 2009, with 23.9% out of a total 1579 clinical trials (Michael, 2009), especially for cancer gene therapy using intratumoral injection (Shirakawa, 2008).

Over the years, Ad vectors have been passed through a serial of evolution mounting evidence of anti-Ad immunological responses (both innate and adaptive) prompted the development of improved Ad versions (Hartman *et al.*, 2007). The first generation adenovirus vectors were developed by deleting one or two early genes, E1 and/or E3. This vector induced strong host immune responses that rapidly remove transgene expression, and thus gene expression became

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low and could only offer transient gene expression (Cao *et al.*, 2004). Newer Ad vectors have improved the ability to persist *in vivo*, facilitating the avoidance of adaptive immune responses (Hartman *et al.*, 2007). An additional deletion in the early genes, E2 and/or E4 was manipulated for the second and third generation vectors (Zhou *et al.*, 1996). Meanwhile, toxicity was found to be reduced in animal models with these vectors (Lusky *et al.*, 1998; O'Neal *et al.*, 1998; Andrews *et al.*, 2001). The deleted all viral genes' helper-dependent adenoviruses, which contain solely the cis acting elements, were the most advanced vector that has been developed (Morsy and Caskey, 1999). These Ad vectors improve the prospects for a long-term gene therapy (Morsy and Caskey, 1999).

In March 2003, 63.4% of all the gene therapy clinical trials were for cancer according to the Journal of Gene Medicine (Clare *et al.*, 2003). Two adenoviral-based gene therapeutics for cancer treatment have been commercialized in China (Peng, 2005; Yu and Fang, 2007). These two constructs have been used as alternative treatments for cancer, in combination with chemotherapy in China (Shirakawa, 2008). This article summarizes the use of adenovirus in the preclinical and clinical trials of cancer gene therapy.

ADENOVIRUS BASED GENE THERAPY IN HUMAN CANCER

Colon Cancer

Colon cancer is the third most frequent cancer for both men and women in Malaysia, which accounts for 7.8% and 5.6% respectively for the two genders (NCR, 2002). The main treatment for colon cancer is surgical resection of the entire tumour, but the result often remains unsatisfactory due to the metastases of the tumour (Faye *et al.*, 2000). Thus, gene therapy may provide an alternative approach to the conventional treatment.

In particular, p53 protein acts as a multifunctional regulator for the cell cycle that is capable of causing apoptosis and becoming tumour suppressor (Levine, 1997). Harris *et al.* (1996) have used adenovirus encoding wild type TP53, a gene that encoded p53 protein, as their construct for their colon cancer study. The researchers found a complete regression and a doubling survival time as compared to the control mice model with intratumoral injection of the construct to the subcutaneous implant p53-mutated colon cancer mice model. A phase I clinical trial, with this construct, showed no tumour regression but with the combination with chemotherapy, those patients revealed partial responses to the treatment (Veenok *et al.*, 1998).

Meanwhile, anti-tumour effects were shown in the murine model with liver metastases colon cancer cell line transduced with adenovirus encoding IL-12 (Caruso *et al.*, 1996). There was a synergistic effect found in a combination treatment of adenovirus encoding TP53 and chemotherapy in immunodeficiency mice (Ogawa *et al.*, 1997).

Carcino-embryonic antigen (CEA) is a cell surface protein that was presumed to play a role in cellular adhesion. Richards *et al.* (1995) found the over-expressing of CEA in 90% of the colon cancer cells, but this was only at a low level in the normal cells. Lan *et al.* (1996: 1997) have developed adenovirus with CEA promoter to provide tumour specific transgene expression. However, the researchers found that the gene expression of this modified vector was weaker compared with the non-specific cytomegalovirus (CMV) promoter. With that, the development of tumour specific vector that is capable of maintaining the transgene expression remains challenging.

Lung Cancer

Lung cancer is the most common cancer among Malaysian men, with 13.9% of all the male cancer patients in the year of 2002 (NCR, 2002). Besides, lung cancer is also in the list of the most

frequent cancers in women, which was ranked at number six, with 4.3% of all the women cancer patients in 2002 (NCR, 2002). Thus, it is important to find an alternative treatment for lung cancer other than the conventional treatment that is available at present. The progressively delineated of the molecular biology of lung cancer, over the past three decades, has allowed the development of lung cancer gene therapy towards targeting (Toloza, 2005). Adenoviruses, with a tropism for lung cancer cells (Stratford-Perricaudet and Perricaudet, 1994), have gained an advantage as a gene therapy vector for lung cancer.

Swisher *et al.* (1999) designed a Phase I clinical trial using adenovirus encoding wild-type TP53 cDNA for advanced non-small-cell lung cancer (NSCLC) after finding a tumour regression in the animal model, following the intratumoral injection of the construct. From this study, the authors concluded that repeated intratumoral injection of the E1-deleted replication-defective recombinant adenovirus containing wild-type TP53 was well tolerated, while transient transgene expression was found to mediate the patients' anti-tumour activity. Besides, vector-related adverse events were also found to be minimal.

The targeting approach for lung cancer, with the use of adenovirus vector such as adenovirus vector with double expression cassette consisting of secretory leukoprotease inhibitor promoter gene that is highly expressed in almost all NSCLCs and CMV promoter (Maemondo *et al.*, 2004), adenovirus-mediated herpes simplex virus thymidine kinase (Fukunaga *et al*., 2002) and polylysine coated adenovirus encoding proto-oncogenec-kit (Schwarzenberger *et al.*, 1996) have shown a promising result for lung cancer treatment.

Breast Cancer

Breast cancer is the most frequent cancers among the women in Malaysia (NCR, 2002). According to the NCR database, 30.4% of the females in Malaysia were newly diagnosed breast cancer patients in the year 2002. A Malaysian woman has 1 in 19 chances of getting breast cancer in her life time (NCR, 2002). Therefore, it is crucial to develop a gene therapy treatment for breast cancer due to the limited success of conventional treatment (Zhang *et al.*, 1996).

Zhang *et al.* (1996) developed an adenovirus containing human interferon consensus gene and tested it on human breast cancer (MDA-MB-435) implanted nude mice. A complete regression, accompanied by the decreased of p53 gene expression, was found in this study. They also found a partial regression of the tumour in the control group, based on which, they concluded that the rapid regression of the breast tumour was due to the combination of the virus oncolysis and the effectiveness of the interferon gene therapy.

An alternative product from the family of cyclin-dependent kinase inhibitors, human INK4a/ARF (alternative reading frame) locus, $p14^{ARF}$, has been identified as a potent tumour suppressor for both *in vitro* and *in vivo* (Serrano *et al.*, 1996; Kamijo *et al.*, 1997; Sherr, 2000). Overexpression of $p14^{ARF}$ will result in cell cycle arrest (Quelle *et al.*, 1995; Kamijo *et al.*, 1997; Weber *et al.*, 2002) and apoptosis (Radfar *et al.*, 1998; Yang *et al.*, 2000; Hemmati *et al.*, 2002). Deng *et al.* (2002) constructed a recombinant adenovirus expressed human $p14^{ARF}$ cDNA for the treatment of human breast cancer. They found an increase in the number of cells in the G_0/G_1 phase but a decrease in the S and G_2/M phases during the time-course study. In addition, there was an increased amount of p53 at the same time. Besides, the findings of their study also indicated a significant increase in the sensitivity of the cells to chemotherapy drug upon $p14^{ARF}$ recombinant adenovirus expression.

A study that evaluated the infection efficiencies of three most promising vectors, namely human adenovirus type 5, canine adenovirus type 2, and human adeno-associated type 2 vectors, was carried out by Lucas *et al.* (2003) in breast cancer cell lines. Meanwhile, the real-time PCR, flow cytometry and antibody blocking studies achieved an agreement in this study that coxsackie adenovirus receptor (CAR) or αv integrin levels are not the main influence to the infection

efficiencies of human adenovirus vector and canine adenoviral vector. They concluded that human adenovirus vector served as the best choice of carrier for gene therapy as compared to canine adenovirus vector and human adeno-associated vector due to the excellent infection efficacy of the human adenovirus vector. This was supported by a study by Mountain (2000), in which a majority of more than 3500 patients who had been enrolled in cancer gene therapy trials approaches were found to have used human adenovirus.

Prostate Cancer

Prostate cancer ranks as the sixth most frequent cancers among men in Malaysia and this accounted for 5.7% in 2002 (NCR, 2002). As for males above the age of 70 years, prostate cancer becomes the top two most common cancers (NCR, 2002). In United States, prostate cancer is the second leading cause of cancer deaths in men (ACS, 2005). The slow growth rate of the prostate cancer as compared with other solid tumour (Schmid *et al.*, 1993) caused no major improvement in patient's survival with chemotherapy (Visakorpi *et al.*, 1991; Kallioniemi *et al.*, 1991). Gene therapy might be an alternative treatment choice for prostate cancer or in combination with conventional therapy to prolong the survival rate of prostate cancer patients.

Tissue- or disease-specific treatments have gained advantages for prostate cancer in gene therapy. The were an estimated 200 prostate-specific genes available for transcriptionally targeting prostate cells (Xu *et al.*, 2001; Nelson *et al.*, 2000). Meanwhile, cancer-specific gene mutation such as p53 (Navone *et al.*, 1993; Bookstein *et al.*, 1993; Brooks *et al.*, 1996) added advantage to prostate-targeting therapy. Adenovirus vector provides a highly tailored therapy that gives different qualities to prostate cancer cell (Lupold and Rodriguez, 2005). Promising results have been reported in several studies, involving the use of oncolytic and suicide gene therapy strategies (Lupold and Rodriguez, 2005). In particular, oncolytic virotherapy uses adenovirus that selectively kills cancer cells by competent replication in tumour cells, but not in normal cells (Hemminki *et al.*, 2003). Thus, a strong cytolytic effect is achieved by oncolytic virotherapy that targets cancer cells (Meerani and Yang, 2010). On the other hand, adenovirus can be used as a vector in suicide gene therapy to deliver non-toxic gene or prodrug to the cancer cells, which eventually triggers the maximum therapeutic effect with limited systemic toxicity (Yazawa *et al.*, 2002). A combination of radiation therapy with replicating adenoviruses shows synergistic effects to radiation and this study is in phases I and II of clinical trials (Freytag *et al.*, 2007).

Ovarian Cancer

Ovarian cancer is the fourth most common cancers among women in Malaysia, and this constituted about 5.0% of the total female cancer (NCR, 2002). Ovarian cancer is also the fourth leading cause of gynaecology malignancy deaths among the female population (Greenlee *et al.*, 2001). The lack of effective screening strategies and the unavailability of clear symptoms at the early stage of the disease caused 70% of the women to be at the advanced stage at the time of get initial diagnosis (Barnes *et al.*, 2002). This leads to the low long-term survival rate over the past 20 years even when advanced surgical technique and chemotherapy are available (Barnes *et al.*, 2002). With that, more advanced gene therapy might be a choice to increase the survival rate of ovarian cancer patients.

The deficiency of coxsackie adenovirus receptor (CAR) in ovarian cancer cells has caused a relative resistance of ovarian cancer cells to adenovirus infection (Vanderkwaak *et al.*, 1999). Retargeting adenovirus receptor to a common receptor of ovarian cancer cells is crucial to improve the tropism of the adenovirus vector to ovarian cancer cells. Wickham *et al.* (1993) added RGD peptide sequences into the penton base with the secondary host cell receptor integrins. Meanwhile,

Vanderkwaak *et al.* (1999) found the RGD-modified adenoviral vector to have shown a promising gene expression to primary ovarian cancer cells compared to human mesothelial tissue.

Cervical Cancer

Cervical cancer was ranked among the most common cancers in Malaysian female population in 2002. The cancer of cervix uteri is the second most common among Malaysia women, comprising of 12.0% of the total female cancers (NCR, 2002). Malaysia had higher age-standardized incidents of cervical cancer as compared to western countries and other countries in Asia (NCR, 2002). Nonetheless, the reason for the high age-standardized incidents in Malaysia remains unknown and it requires further investigation.

Human papillomavirus (HPV) has generally been recognized as the main contributor to cervical cancer. Hamada *et al.* (1996) found that the recombinant adenoviral incorporated wild type p53 was a potential therapy for HPV-positive cervical cancer cells. Further evaluation by Woong *et al.* (2002) indicated significant cell growth suppression by p53 recombinant adenovirus in HPV 18-infected cells (HeLa and HeLaS3) as compared to HPV 16-infected cells (CaSki and SiHa). Besides, the researchers also concluded that different cancer cell lines would have different cell cycles arrest phases and different roles of cell growth suppression through apoptosis in the event of over-expression of wild type p53 in those cell lines.

Table 1 summarizes the clinical trials in cancer gene therapy involving adenoviral vector. Adenovirus is the most used vector for cancer gene therapy in those trials.

ADENOVIRAL-BASED GENE THERAPY FOR ANIMAL CANCER

Canine

Biological and environmental similarities among dogs and humans have made dogs one of the appropriate preclinical models for gene therapy (Kruth, 1996). Andrawiss *et al.* (1999) studied the potential of adenoviral gene transfer in dog prostate, aiming that dog as a basic preclinical model for human prostate cancer gene therapy. The researchers found transgene expression in prostates and epithelial cells with no side effects on the dogs.

Meanwhile, Von Euler *et al.* (2008) have reported the usage of adenoviral vector for the gene therapy in canine malignant melanoma. Two dogs with oral and conjunctiva malignant melanoma respectively were given recombinant adenovirus encoding human CD40L gene as a treatment. CD40L protein is a member of the tumour necrosis factor (TNF) that is mainly expressed on activated T cells. The dog with oral melanoma was in stage III at the time it was diagnosed. This dog showed a complete regression of melanoma after 2 intratumoral injections of recombinant adenovirus. However, no recurrence of the tumour and no abnormality were found after the gene therapy. The other dog with conjunctival melanoma was in stage I with a rapid progression. This dog was treated with the same recombinant adenovirus just like the dog with oral melanoma but it was given 6 injections over 60 days. The tumour regressed dramatically within 60 days of post treatment with no sign of progression or metastasis. Adenoviral immunotherapy was efficient for canine melanoma and could be considered for human melanoma treatment, especially when aggressive surgical excision shortens the survival life span (< 10 months) (Dow *et al.*, 1998; MacEwen *et al.*, 1999).

Canine osteosarcoma is the most common cancer in large dogs, with over 8000 cases in Unites States annually and there is no curative treatment for this particular disease at the moment (Hemminki *et al.*, 2003). Due to the unlikely replication of human adenovirus in canine cells, Hemminki *et al.* (2003) have generated the first non-human oncolytic adenovirus. They found that

TABLE 1
The use of adenoviral vector for clinical trials in cancer gene therapy

Gene	Route	Phase	Tumour type	No. of patients
IL-2	ex vivo/ s.c.	I	Stage IV melanoma	15
IL-2	Intratumor	I	Stage IV melanoma	23
IL-2	Intratumor + prostectomy	I	Localized prostate cancer	12
IL-2	Intratumor	I	non-small-cell lung cancer (NSCLC)	21
p53	Intratumor	I	Advanced NSCLC	21
p53	Intratumor + chemotherapy	I	Advanced NSCLC	24
p53	Intratumor	I	Advanced NSCLC	15
p53	Intratumor + chemotherapy	II	Advanced NSCLC	25
p53	Intratumor or intravesical instillation+ cystectomy	I	Bladder cancer	12
p53	Intravesical instillation	I	Bladder cancer	13
p53	i.p. + chemotherapy	I/II	Recurrent ovarian cancer	36
p53	Intratumor	I	Advanced NSCLC	27
p53	Intratumor + radiotherapy	II	Advanced NSCLC	19
p53	Intratumor + surgery	I	Recurrent glioma	15
Anti-erbB2 single chain antibody	i.p.	I	Ovarian cancer	15
HSV-TK	Intratumor	I	Recurrent glioblastoma	13
HSV-TK	Intratumor	I/II	Recurrent glioblastoma	14
HSV-TK	Intratumor + surgery	I	Recurrent glioblastoma	14
HSV-TK	Intratumor	I	Prostate carcinoma	18
HSV-TK	Intratumor + radiotherapy	I/II	Prostate carcinoma	30
HSV-TK	Intratumor	I	Prostate carcinoma	11
*dl*1520	Intratumor	I	Recurrent head and neck cancer	22
*dl*1520	Intratumor	I	Hepatocellular carcinoma	3
*dl*1520	Intrahepatic artery or iv	I	Colon cancer liver metastases	6
*dl*1520	Intrahepatic artery+ chemotherapy	II	Liver metastases from colon cancer or unknown primary	7
*dl*1520	i.v. on day 1 and intratumor on subsequent days	II	Hepatocellular carcinoma	5
*dl*1520	Intratumor	I	Pancreatic carcinoma	23
*dl*1520	Intratumor + chemotherapy	I	Metastatic solid tumours	10
*dl*1520	i.p.	I	Advanced ovarian cancer	16
*dl*1520	Intratumor + chemotherapy	I/II	Advanced pancreatic carcinoma	21
*dl*1520	Intratumor + chemotherapy	II	Recurrent head and neck cancer	37
*dl*1520	Intratumor	II	Head and neck cancer	40
*dl*1520	Intrahepatic artery+ chemotherapy	II	Gastrointestinal carcinoma metastasis to the liver	27
CN706	Intratumor	I	Recurrent prostate cancer	20
Ad5-CD	Intratumor	I	Recurrent prostate cancer	16

(Obtained and modified from Baron *et al.,* 2004. Endocrine aspects of cancer gene therapy. *Endocrine Reviews*, *25*(1), 1-44)

this virus could effectively kill the primary canine osteosarcoma cells from a dog that underwent osteosarcoma surgery. Besides, the advantage of the *in vivo* therapeutic was attained from the development of this conditionally replicated canine adenovirus (Hemminki *et al.*, 2003).

A preparation study for phase I clinical trail of the recombinant adenoviral gene therapy for locally recurrent prostate cancer was carried out by Dwyer *et al.* (2005). This study showed no vector-related toxicity and the successful introduction of gene expression in the prostate gland of dogs. More importantly, no animal experienced surgical complications and no significant change was shown in the serum chemistry panels following the therapy. These results provide insight for further translation of this experiment into clinical setting.

Lung, colon or breast cancer in canine is not a good model for human cancer. This is due to the low prevalence of these cancers in dogs and the biologically difference of these organs between dog and human (Kruth, 1996).

Feline

Siddiqui *et al.* (2007) conducted a study using adenovirus harbouring feline interleukin-12 (IL-12) for feline soft tissue sarcomas gene therapy. The study was in phase I clinical trial. Thirteen cats with confirmed diagnosis of sarcoma underwent a gene therapy using recombinant adenovirus following a prior radiation therapy of 22 days. The recombinant adenovirus cloned feline IL-12 was intratumorally injected and the tumour was heated at 24-hours post-injection. This clinical trial was carried out to check the systemic toxicity and tumour expression of IL-12. From this study, Siddiqui *et al.* (2007) found that hyperthermia-induced gene therapy capable of localizing gene expression and limiting systemic toxicity.

The successes in using adenovirus system for preclinical or clinical study in canine and feline species have provided future insight into the development of cancer vaccine for animals. However, the unlikely replication of the human adenoviral vector in canine and feline cells might reduce the efficiency of vector. Therefore, a study on Ad dosage is crucial to inhibit tumour progression or enhance tumour regression effectively. Besides, the limited number of canine or feline in those trials might influence the validity of data. More trials are required for validating the use of the Ad vector as a carrier for cancer gene therapy in canine or feline.

Hamster

Recombinant hamster interferon (IFN)-α adenovirus can effectively suppress hamster pancreatic tumour growth in Syrian hamster (Hara *et al.*, 2007). Tumour regression was discovered in both the injected subcutaneous tumours and untreated tumours in the peritoneal cavity and at distant sites. No significant difference was found in the systemic toxicity among the treated and untreated groups. Thus, local IFN- α gene therapy has been proven to be a promising therapeutic strategy for pancreatic cancer (Hara *et al.*, 2007).

Another study that made use of the Syrian golden hamster as a biliary cancer model was conducted by Kim *et al.* (2006) who used genetically modified bone marrow stromal cells containing adenoviral harbouring human interleukin-2 (IL-2) gene as a treatment. All the hamsters in the treatment group survived with no evidence of disease during the 12 weeks' observation period. However, the hamsters in the untreated and control group showed disseminated metastases that involved lungs as early as 4 weeks. Thus, the researchers made a conclusion that adenovirus vector carrying IL-2 as an effective treatment for biliary cancer.

CONCLUSIONS

The advancing field of gene therapy promises survival remedies for the ever raising number of cancer patients worldwide. Moreover, innovative and combinatory treatment of Ad gene therapies with the aid of the advanced molecular biology tools have shown promising results in the treatment of cancer. Meanwhile, a continual evaluation on the specificity, transgene expression and safety of Ad gene therapy in cancer will ensure feasible approach for successful outcomes.

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