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Review

Biological delivery approaches for gene therapy: Strategies to potentiate efficacy and enhance specificity

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ABSTRACT

Nowadays many therapeutic agents such as suicide genes, anti-angiogenesis agents, cytokines, chemokines and other therapeutic genes were delivered to cancer cells. Various biological delivery systems have been applied for directing therapeutic gene to target cells. Some of these successful preclinical studies, steps forward to clinical trials and a few are examined in phase III clinical trials.

In this review, the biological gene delivery systems were categorized into microorganism and cell based delivery systems. Viral, bacterial, yeast and parasite are among microorganism based delivery systems which are expanded in this review. In cell based approach, different strategies such as tumor cells, stem cells, dendritic cells and sertoli cells will be discussed.

Different drawbacks are associated with each delivery system; therefore, many strategies have been improved and potentiated their direction toward specific target cells. Herein, further to the principle of each delivery system, the progresses of these approaches for development of newer generation are discussed.

1. Introduction

Gene therapy describes the use of exogenous DNA for therapeutic agent. The application of exogenously administered genes now are used in a wide variety of approaches including immunomodulation, genetic vaccination and genetic pharmacology (Rao et al., 2007).

To induce successful therapeutic responses in gene therapy, vectors should be able to generate high levels of transgenic proteins at target cells to decrease the risk of toxicity at other sites than target cells. The major focus of this review is on the progress of biological delivery systems to enhance their direction into target cells and also to reduce their delivery to other cells.

In this review, the biological gene delivery approach was categorized into microorganism and cell based delivery system. In microorganism based approach, viral, bacterial, yeast and parasite based delivery systems will be discussed. The cell based delivery system is divided into tumor cell, DC and stem cell based approaches.

2. Therapeutic strategies in cancer gene therapy

In cancer gene therapy, further to antigen delivery in vaccines several other approaches can be used such as mutation correction; enhancement of the immune response against tumor cells, RNA interference, targeted lysis of tumor cells using selective replicative viruses, anti-angiogenic and suicide gene therapies.

Suicide gene therapy is based on the introduction of a viral or a bacterial gene, which converts a non-toxic compound to a lethal drug, into tumor. Among the large number of suicide systems, the herpes simplex virus thymidine kinase gene (HSV-tk) with ganciclovir (GCV), and the cytosine deaminase gene (CD) of *Escherichia coli* along with 5-fluorocytosine (5-FC) are the most extensively studied. GCV is a non-toxic agent which is converted to toxic drugs by phosphorylation *via* viral thymidine kinase. In CD/5-FC system, CD converts the non-toxic antifungal agent 5-FC into 5-fluorouracil (5-FU) (Duarte et al., 2012).

Inhibition of angiogenesis has been emerged as a new strategy in the field of cancer gene therapy. There are many heterogeneous angiogenesis inhibitors which differ in their origin and potency. Pre-clinical studies indicated that long-term administration is required to obtain prolonged anti-tumor effects. Therefore, their delivery by a gene therapy approach seems to be effective (Persano et al., 2007). Furthermore, inhibiting multiple angiogenic pathways with prolonged, sustained levels of multiple therapeutic agents could be easier to utilize rather than repeated, systemic

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boluses of numerous anti-angiogenic agents (Tandle et al., 2004). Over the last decade, eight anti-angiogenic agents were approved by United States Food and Drug Administration (FDA) for cancer treatment and many other anti-angiogenic agents are in phase III clinical trials (Duda, 2012).

Another approach for improving immune stimulation is cytokine gene therapy. The key issues in this strategy are using optimal cytokine, efficient and durable cytokine expression system (Liu et al., 2010). Cytokine gene therapy is associated with local expression, enhanced anti-tumor activity and reduced toxicity (Qian et al., 2006).

Many novel strategies including oncolytic virus expressing cytokine and cytokine expressing vectors under the tumor-specific promoters are on progress. Furthermore, some chemokines entered different clinical trials phase as cancer therapy with various delivery systems. For example, a phase I clinical trial of vaccination with IL-2 and lymphotactin secreting genetically modified neuroblastoma, reveals excellent tolerance with little toxicity (Rousseau et al., 2003). Here, we are describing some of the recent approaches in more details.

3. Microorganism based delivery system

There are various applications for using viruses, bacteria, yeast and parasite as gene delivery system which as discussed in separate sections.

3.1. Viral based approach

The most common viruses in the field of gene therapy are retroviruses, adenoviruses (Ads) and adenoassociated viruses (AAVs). The major advantage of viral vectors is their high gene delivery efficiency. Each virus has its own advantages and disadvantages, which make them applicable in a special therapeutic setting. The toxicity of viral protein in normal tissue, the larger size of viral vectors than the space between fibers in extracellular matrix, the possibility of random integration of the vector DNA into the host genome and their high cost are challenging aspects of therapeutic genes delivery using viral vectors. Comparing with other immunotherapy strategies, recombinant viruses can be produced, administered and quality controlled more easily. However, host-induced neutralizing antibodies as well as tissue inflammation and injury caused by innate and adaptive host responses to Ad vectors and premature clearance of Ad-transduced cells limit their successive use (Larocca and Schlom, 2011).

Viral vectors can be applied either to deliver the transgene *in vitro* (*ex vivo* gene therapy) or can be directly administrated to patients (*in vivo* gene therapy) (Liu et al., 2010).

In *in vivo* gene therapy, replication-deficient viruses are applied as vectors for transferring therapeutic genes into target cells. In another strategy, named virotherapy (viral oncolysis), recombinant viruses are used for targeted cancer treatment. In virotherapy, killing of tumor cells are caused by selective viral infection, replication, cell lysis, release and spread of progeny viruses that infect and kill neighboring cancer cells (Dorer and Nettelbeck, 2009).

3.1.1. Adenovirus based vector

The ease of infection with the large DNA capacity make adenovirus based vector very popular (Edelstein et al., 2007). Infection of both dividing and non-dividing cells, high levels of transgene expression, ability to grow to high titers *in vitro*, lack of integration into host genome, physical and genetic stability make these vectors particularly attractive. Adenoviruses infect DCs, up-regulate co-stimulatory molecules, and elicit cytokine and chemokine responses, thus effectively present antigens and induce potent immunity (Robert-Guroff, 2007).

Due to the existence of antibodies against adenoviruses in most people, the adenovirus reinjection would be problematic (Appledorn et al., 2008). In an attempt to solve this problem, heterologous prime-boost regimen, delivering of the same antigen using different vectors, was applied. In this regimen, the first viral vector-specific T cells are not boosted and greater numbers of the antigen-specific T cells are expanded. Vaccinia virus and adenovirus showed particular efficacy at boosting immune responses (Anderson and Schneider, 2007). Prostvac^{TM} (BN ImmunoTherapeutics) consists of a recombinant vaccinia vector as a primary vaccination, followed by multiple booster vaccinations with recombinant fowlpox vector. Both priming and boosting vectors encode prostate-specific antigen (PSA) as well as a multiple T-cell costimulatory molecules (Madan et al., 2009).

3.1.1.1. First and second generation Ad vectors. It was found that the expression of early genes (E) caused mature adenoviral progeny production. The Ad vectors with deleted E1, with or without E3 deletion, are designated as first generation vectors. Deletion of E1 led to replication deficient viral vectors that were propagated in helper cell lines (Trapnell and Gorziglia, 1994). The first generation Ad vectors carried ~8 kb foreign genes. Recombination with the viral DNA sequences present in the complementing cell line may result in replication competent adenoviruses (RCA). The possibility of uncontrolled replication of this RCA contaminant in the patient increases the safety considerations (Saini et al., 2007).

The vectors with deletions in E2 and E4, accompanied by E1 and E3, are second generation Ad vectors with a transgene capacity of ~14 kb. These deletions resulted in reducing the host immune response, increasing the vectors capacity, and preventing generation of replication-competent adenoviruses during amplification, thereby increasing their safety profile (Alba et al., 2005). Proteins encoded by E2 and E4 regions are essential for replication in cell culture. Therefore, these are provided *in trans* by respective producer cell lines based on HEK293 cells (Wang et al., 2005). However, vector-related toxicity and immunogenicity related to the expression of the remaining viral genes are still the major obstacles for usage of these vectors (Fang et al., 1996).

3.1.1.2. Gutless vector. To further reduce the immunogenicity and improve the safety of Ad vectors, the gutless vector system with the transgene capacity of ~36 kb has been developed. These vectors are devoid of all viral genes except those that are required for packaging and replication (Wang et al., 2005). To produce gutless Ad vectors (as demonstrated in Fig. 1A) helper virus for supporting DNA replication and packaging is required. As the packaging signal is deleted in helper virus, it is restricted for packaging into viral particles. This strategy cannot completely eliminate helper viruses in the final purified gutless vector production. To remove contamination of helper virus from the final preparation, different systems based on the excision of the helper-packaging signal have been generated. Among them, Cre-loxP system is mostly used, although contamination levels still are 0.1–1%, too high to be used in clinical trials (Alba et al., 2005). The Cre-loxP system is demonstrated in Fig. 1A. Many strategies were tried to further improve the gutless Ad vector system by modification of the Cre/loxP recombination system or by use of different recombination system, such as FLP/frt system. However, the strategies based on the deletion of the packaging signal in the helper virus DNA, are the current most efficient way to produce gutless Ad vectors.

As capsid proteins of viral vectors stimulate the immune response, systemic administration of gutless viruses also induces the immune response. In addition, production of neutralizing antibodies also prevents re-administration of the same serotype vectors. Since gutless vectors do not express any viral genes after the initial inflammation, the transduced cells are not recognized