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A Panoramic View on Gene Therapy: Applications and Complications

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Abstract

Gene therapy can be considered as emerging therapeutic technology which can be used to handle various diseases. The review emphasizes the panoramic view on gene therapy, which includes the various methods for implanting or replacing corrected genes into the human body using specific vectors carrying the target gene. The primary area of advancements in the field of gene therapy is concerned with the use of viral vectors as transport mechanism to implant modified gene into the target cells in which the most widely used viral vectors for the therapy are adenovirus and retrovirus. Additionally, there are also several alternative methods to inject the gene into the cell, including non-viral vectors such as gene gun. The study also characterises the applications of the gene therapy in non-genetic diseases such as cancer, hypertension and ischemia along with the complications and ethical view of gene therapy.

Keywords: Gene Therapy, Cancer, Retrovirus, Hypertension

INTRODUCTION

Gene therapy is the medical advancement developed for the treatment, cure or prevention of specific diseases. Gene therapy includes insertion of modified nucleic acid (DNA or RNA) into the human cell with the help of various vectors (Kaufmann, K.B. *et al*., 2013). Gene therapy can be classified into *ex-vivo* and *in-vivo* gene therapy. *Ex-vivo* is concerned with the transfer of gene in cell culture and the *in-vivo* process is concerned with direct transfer of modified gene into the human body. It can be given by mainly intra-muscular and intracerebral injections. Gene therapy can also be characterized into 2 different terms awhich are somatic and germline gene therapy (Wirth, T. *et al*., 2013). In somatic gene therapy, the modified gene will not continue through generations. On the other hand, germline gene therapy passes the modified gene into future generations (Wirth, T. *et al*., 2013). Gene therapy can utilize either viral vectors or non- or non-viral vectors. Viral vectors are the most widely used type of vectors in gene therapy and it is mainly concerned with gene transduction (Gardlík, R .*et al*., 2013). On the other hand, non-viral vectors are associated with the transformation process (Gardlík, R. *et al*., 2013). Viral vectors used in the gene therapy are retrovirus, adeno-associated virus and herpes simplex virus in which adeno-associated virus (AAV) is most widely used vector for the monogenic disorders like sickle cell disease and Huntington disease (Gardlík, R. *et al*., 2013). Similarly, for non-viral vectors, the electroporation technique is the latest advancement of gene therapy. The most important gene therapy innovation has so far been based on cancer treatment. Cancer is one the most difficult disease to treat and in future, gene therapy can be the most phenomenal technique to put the end on the severity of cancer. There is still a number of undiscovered vectors and advancements of gene therapy which are into the clinical trials.

In-situ, *in-vivo* and *ex-vivo* gene therapy

In-situ gene transfer is the transfer of the corrected gene into the specific organ or tissue. According to the literature, *In-situ* gene transfer method can be used with an antineoplastic prodrug such as Ganciclovir (Wildner, O. *et al*., 1999).In this treatment, the corrected gene is first applied to the target cell by in-situ gene transfer who will increase the efficacy of Ganciclovir towards target cell (Wildner, O. *et al*., 1999). *In-vivo* gene therapy is the genetic transfer method in which the corrected gene is transferred to the subject systemically by injection.

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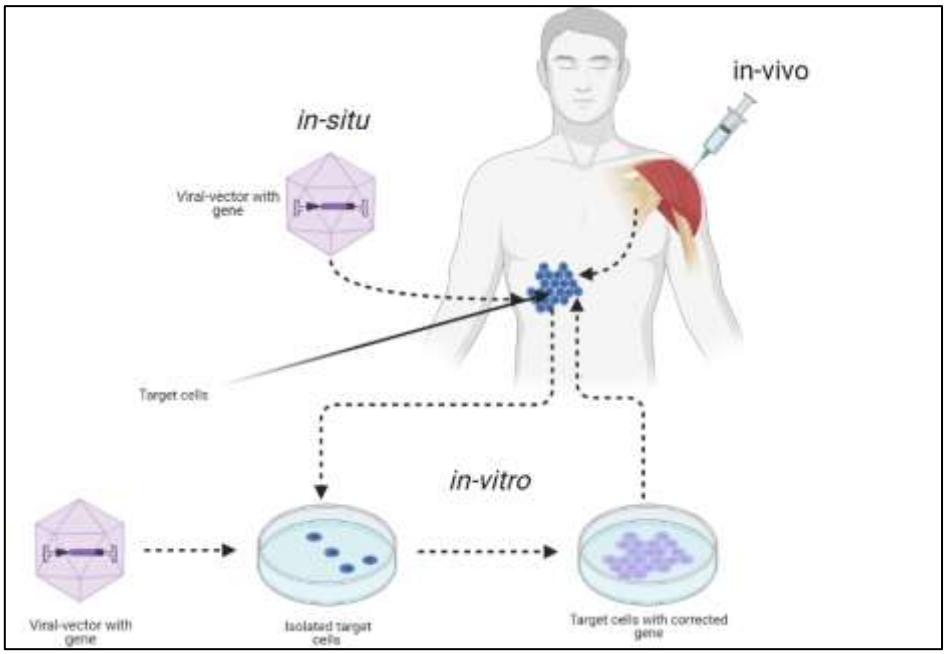

Figure 1 Ex vivo, in vivo, and in situ gene therapy

The major difference between *in-situ* and *in-vivo* gene therapy is that in the case of the in-situ process the corrected gene is directly transferred to the target (Dunbar, C.E. *et al*., 2018). On the other hand, in the *in-vivo* process, the corrected gene is being applied to the systemic circulation (Dunbar, C.E. *et al*., 2018). The major concern related to *in-vivo* gene therapy is inefficient targeting. The gene transferred to the circulation end up by activating the immune system (Van Haasteren, J. *et al*., 2018). Viral vectors used in *in-vivo* gene therapy may induce production of antibodies against the particular viral factor and generated antibody will not allow the gene transfer (Van Haasteren, J. *et al*., 2018). Hence, the viral factor of the vector must be excluded from the vector or the neutralizing antibody can be reduced from the subject body (Van Haasteren, J. *et al*., 2018). Two approaches discussed above may improve the delivery efficacy of gene therapy and these approaches are now the main concern of gene therapy research field. There are several ways to transfer the corrected gene by *in-vivo* gene therapy which includes direct injection of DNA liposome by system infusion, injecting DNA plasmid using biolistic gene gun or tissue injection of recombinant virus containing corrected gene (Nayerossadat, N. *et al*., 2012).

Ex-vivo gene transfer is the type in which the corrected gene is being transferred to the target cell of the subject in cell culture to produce a modified cell with the corrected gene of choice (Nayerossadat, N. *et al*., 2012). The step-in *Ex-vivo* gene transfer is the transplantation of the modified cell into the subject body to replace the defective cell or to start the production of therapeutic proteins (Gowing, G. *et al*., 2017). The *ex-vivo* method allows the in-depth modification of the cell with a particular gene (Gowing, G. *et al*., 2017).

Another advantage of *Ex-vivo* method is that the correct gene or viral vector do not get into contact with the systemic circulation. Hence, the negative effect of the neutralizing antibodies is minimal in *Ex-vivo* method. According to the literature, Ex-vivo gene therapy demonstrated significant results in Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and stroke.

Vectors

Gene therapy is the process of injecting corrected DNA into the subject cell. Although, direct injection of delivery of the same is less efficacious. Henceforth, there are several molecules and techniques which can be considered to improve the efficacy of gene therapy (Robbins, P.D. and Ghivizzani, S.C. 1998). These molecules are known as a vector. Vectors are best described as any molecule or system providing functional environment and protection to the DNA and facilitates transfer and delivery of a gene to the target cell of the host (Robbins, P.D. and Ghivizzani, S.C. 1998).

Two types of vectors are mainly associated with gene therapy which are viral vectors and non-viral vectors.

Viral vectors

Different kinds of viruses can be used as vectors in gene therapy. Viruses are known for their best ability to penetrate and proliferate into the human cell (Robbins, P.D. and Ghivizzani, S.C. 1998). The concept can be used to insert corrected DNA into the target cell. Before using the virus as a vector, it is modified accordingly to remove the viral infectious part of it and the modified DNA is inserted into it. The vector is injected through *in-vivo* or *ex-vivo* method into the systemic circulation or to the target cell (Robbins, P.D. and Ghivizzani, S.C. 1998). Based on several studies, the infectious viral factor cannot be removed completely (Robbins, P.D. and Ghivizzani, S.C. 1998). During the recombination process, the eliminated viral gene can be received by the vector which will end up producing infection and deletion of the therapeutic gene. Vectors with these abilities are known as reactivants (Robbins, P.D. and Ghivizzani, S.C. 1998).

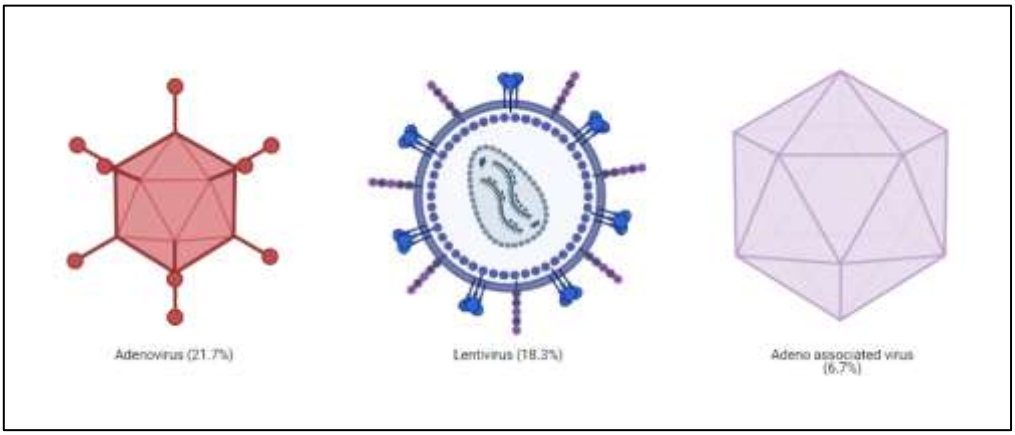

Figure 2 Viral vectors

Viral vectors can be distinguished in two different types which are non-lytic viruses and lytic viruses. The non-lytic viruses will not destroy the host cell and lytic viruses will destroy the infected cell. Despite having the risk of infection, viral vectors are still the most widely used vector for gene therapy (Robbins, P.D. and Ghivizzani, S.C. 1998).

Adenoviruses

Adenoviruses are the most widely used vectors in gene therapy. There are more than 50 serotypes of adenoviruses have been identified in which type 2 and type 5 can be utilized as a vector in gene theerapy (Nadeau, I. and Kamen, A. 2003). The group of adenoviruses are mainly concerned with respiratory diseases is mainly used in gene therapy. The immune system of the subject plays an important role while using adenovirus as a vector as the majority of the human population has been gone through the adenovirus infection and thus already has antibodies produced against it (Nadeau, I. and Kamen, A. 2003). Hence, most of the gene therapy treatment using adenovirus vector will not produce any major infectious side effects and the vector will get degraded within 24 hours (Nadeau, I. and Kamen, A. 2003).

Retrovirus

Retroviruses are RNA viruses which replicate through DNA intermediates. These vectors are the first recognised as the first-ever created viral vectors (Anson, D.S. 2004). Retroviruses are consisting of several genes including LTR, gag, pol and env. long terminal repeats (LTR) are essential for replication. The other genes which are gag, pol and env can be replaced with therapeutic gene and can be used further in gene therapy (Anson, D.S. 2004). removal of gag, pol and env will also remove the infectious property of the virus.

Figure 3 Modification of retrovirus

Hence, it won't produce any infection while using as a vector in gene therapy. Although,

retrovirus vector is found to be less efficient *in-vivo* trials and thus it is mainly used as *ex-vivo* gene therapy (Anson, D.S. 2004). Also, there is one more limitation associated with the retrovirus vector is it is only effective against the proliferating cells and not on dormant cell (Gardlík, R .*et al*., 2013). To overcome this limitation, lentivirus can be used to apply on proliferating as well as dormant cells (Anson, D.S. 2004). Lentivirus also provide long-term gene expression without any inflammatory responses.

Non-viral vectors

Viral vectors are not suitable for the host with lower immunogenicity. Additionally, the infectious effect of viral vectors is yet to overcome completely. Hence, Non-viral vectors are found to be an important way to enhance the safety of gene therapy (Sung, Y.K. and Kim, S.W. 2019). It is a simpler and safer alternative to viral vectors. Non-viral vectors can be defined as any molecule of DNA with the corrected gene which can be used as a vector in gene therapy (Sung, Y.K. and Kim, S.W. 2019). The simplest example of non-viral gene therapy is the use of naked DNA. Intramuscular injection of naked DNA will produce low but significant results without any side effects. Gene-gun is a notable example of non-viral gene therapy (Sung, Y.K. and Kim, S.W. 2019). it is consisting of miniature particles of gold on which the DNA is bound (Sung, Y.K. and Kim, S.W. 2019). These particles are injected under great pressure and speed with the help of helium to cross the membrane barrier (Sung, Y.K. and Kim, S.W. 2019).

Applications of gene therapy

Gene therapy has found to be a dominant tool for the treatment, cure and prevention for the number of diseases which is mainly concerned with treating the cause instead of treating the symptoms.

Cancer

Although gene therapy was initially developed to treat genetic diseases, the majority of gene therapy trials are conducted to treat cancer (Kumar, M. and Misra, A. 2011). The earliest trials of cancer gene therapy focused on inserting a modified gene into the target cell in order to increase the efficacy of antineoplastic drugs; this approach is referred to as suicide gene therapy (Kumar, M. and Misra, A. 2011). Suicide gene therapy is currently only available in combination with herpes vector and ganciclovir (GCV) (Kumar, M. and Misra, A. 2011). Numerous clinical trials for the use of gene therapy in various types of cancer are currently underway. These trials include use of gene therapy against brain cancer, lung cancer, breast cancer and prostate cancer.

Hypertension

Applications of gene therapy to cardiovascular disorders have also been applied. Hypertension is characterised by high blood pressure levels, which can be caused by many polygenic and environmental causes (Phillips, M.I. 1999). This is contributing to many cardiovascular diseases such as heart disease, atherosclerosis, and stroke. Gene therapy for hypertension is mostly directed at the different components of the renin-angiotensin system (RAS) in the brain (Phillips, M.I. 1999). Renin activates the synthesis of angiotensin I (Ang I) from angiotensinogen (AGT), which is then transformed by angiotensin-converting enzyme (ACE) into angiotensin II (Ang II). Angiotensin II acts on angiotensin type I receptors (AT1R) and angiotensin type 2 receptors (AT2R) and causes hypertension.

To cure hypertension, the modified gene strategy can be used by using the antisense oligonucleotide (AS-ODNs) (Phillips, M.I. 1999). It is a modified gene which can be transferred to a specific cell by using vector-like adenovirus and lentivirus to cure hypertension (Phillips, M.I. 1999). According to the literature, AS-ODNs to AT1R reduced blood pressure in various rat hypertension models including spontaneous hypertension rats' model (SHR), cold-induced hypertension model and environmental model and the two-kidney one-clip model (Phillips, M.I. 1999).

Ischemia

Ischemia is the improper blood supply to the tissues. It is furtherly classified into peripheral ischemia which is related to the peripheral tissues and myocardial ischemia which is the insufficient blood supply to the heart. There can be several causes of ischemia including the presence of blood clot or artery spasm. Gene therapy can be used as a substitute therapy to restore natural blood flow (Lavu, M. *et al*., 2011). The basic approach to treat ischemia is known as angiogenesis. It is the physiological process for the formation of new small blood vessels (Lavu, M. *et al*., 2011). Therapeutic

angiogenesis can be targeted by using vascular endothelial growth factor family (VEGF). In Ischemia gene therapy, the naked plasmid with VEGF is incorporated in the target cell with the help of specific vector (Lavu, M. *et al*., 2011). There is another growth factor which can be incorporated through gene therapy to increase blood flow which is known as growth factor family (FGFs) (Lavu, M. *et al*., 2011). This concept is found to be useful in the treatment of ischemia.

Complications of gene therapy

The process is not fully developed yet and it may cause number of complications. The displacement of the inserted gene may result into the DNA mutations or even to the cancer (Pfeifer, A. and Verma, I.M. 2001).The most widely seen complication to the gene therapy is the inflammation due to the immune response (Lowenstein, P.R. *et al*., 2007). Mechanism behind the inflammation is that the body's immune system may attack the virus vectors used in the gene therapy and results into inflammation or even into organ failure (Lowenstein, P.R. *et al*., 2007). Another complication is the infection from viral vectors as complete removal of viral factor is not yet possible (Tomanin, R. and Scarpa, M. 2004). Virus may recreate the gene responsible for the viral factor in host cell and cause serious infection (Tomanin, R. and Scarpa, M. 2004).

Gene therapy is the process of genetical modification of germlines and this possibility has been popular discussion in scientific field from long time (Evans, J.H. 2002). In ethical terms, Gene therapy should be only used as a treatment method to fix the disease condition rather than using it as a tool of enhancement to improve the human species (Bostrom, N. and Roache, R. 2008). Germ line gene therapy is also concerned with ethical discussion of this field. Effects of germ line therapy extend indefinitely into the future (Fletcher, J.C. 1983). Germ line gene therapy is the objected to correct the genetic defect once and for all which also lies into the ethical problem (Evans, J.H. 2002). Insertion of defective gene through germ line therapy will pass to the future generation indefinitely (Fletcher, J.C. 1983). Hence, this process can create a genetic disease throughout many future generations.

Gene Doping is also widely discussed ethical issue connected with gene therapy. According to the world Anti-Doping Agency (WADA), gene doping is the process of injecting non-therapeutic useful genes into the subject to enhance their athletic performance (Unal, M. and Unal, D.O. 2004). This process was found to be used for exploiting the athletic performance of sportsman to enhance their performance (Unal, M. and Unal, D.O. 2004). World Anti-Doping Agency (WADA) banned this process in order to maintain the integrity of sports competition (Unal, M. and Unal, D.O. 2004).

DISCUSSION AND CONCLUSION

The study elucidates the primary aspects of gene therapy which are essential to develop advancements of the techniques for the insertion of gene into the target cell intended to exert therapeutic benefits. Whether to use somatic cell gene therapy or germ line gene therapy is determined by a variety of considerations, including the gene therapy's success rate and the possibility of adverse genetic effects. As the germ line gene therapy carry the modified gene over generations, it may result into the development and spread of serious genetic malfunction. Additionally, the clinical trials of germ line gene therapy will take much longer time compared with somatic cell gene therapy. Hence, somatic cell gene therapy can be considered as safer and favourable than germ line gene therapy as the modification of gene will be limited to the patient. To ensure the safety and accuracy of gene therapy, there are several different ways to inject the corrected genes which are *in-vivo*, *in-vitro* and *in-situ* methods by using viral and non-viral vectors. Advancements of the gene therapy can be studied by using different viral vectors which can be concluded as the major pathway to find novel treatment methods. According to the literature, gene therapy found to give significant benefits in cancer treatment by increasing the efficacy of anti-neoplastic drugs in cancerous cells. Although, there are several limitations and risk factors of the gene therapy which should be taken into considerations as ultimately it is the process of altering the natural genetic makeup of human race. Hence, there is huge side of the gene therapy is yet to be explored.

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