

Review

Gene Therapy and Modification as a Therapeutic Strategy for Cancer

Ahsen Chaudhry¹, Daud Akhtar, BSc(H)¹

¹Faculty of Medicine, University of British Columbia

ABSTRACT

Gene therapy is an exciting new field of personalized medicine, allowing for medical procedures that can target diseases such as cancer in novel ways. Technologies that involve gene transfer treatments allow for the insertion of foreign DNA into tumour cells, resulting in restored protein expression or altered function. Gene therapy can also be used as a form of immunotherapy, either by modifying cancer cells to make them better targeted by the immune system, or by modifying the body's immune cells to make them more aggressive towards tumours. Additionally, oncolytic virotherapy uses classes of genetically modified viruses that can specifically target and interfere with tumour cells. The ongoing development of the CRISPR/Cas9 gene editing tool may also have promise in future therapeutic applications, with the tool being capable of removing cancer-causing, latent viral infections, such as HPV, from afflicted cells. Nonetheless, there are still many questions of safety, efficacy, and commercial viability which remain to be resolved with many gene therapy procedures. There is also emerging controversy over the ethical, legal, and moral implications that modifying the genetic content of human beings will have on society. These concerns must be confronted and addressed if the benefits promised by gene therapy are to be properly realized.

RÉSUMÉ

La thérapie génétique est un nouveau domaine d'étude médicale personnalisée qui permet de cibler des maladies spécifiques comme le cancer de façon innovatrice. Cette thérapie utilise le transfert de gènes avec une insertion d'ADN étrangère dans les cellules cancéreuses dans le but de restaurer l'expression des protéines et de retrouver la fonction cellulaire. La thérapie génétique peut aussi être utilisée comme une forme d'immunothérapie, soit en modifiant les cellules cancéreuses pour qu'elles soient mieux ciblées par le système immunitaire ou en modifiant les cellules immunitaires du corps pour les rendre plus agressives envers les tumeurs. De plus, une virothérapie oncolytique utilise des virus génétiquement modifiés qui peuvent cibler spécifiquement et interférer avec des cellules cancéreuses. Le développement du système d'édition génétique CRISPR/Cas9 s'avère prometteur pour les applications thérapeutiques futures. Cet outil est capable d'enlever les infections virales latentes dans les cellules affectées qui peuvent causer le cancer, tel que l'HPV. Malgré ces découvertes, plusieurs questions importantes demeurent quant à la sécurité et à l'efficacité de leur application. Il s'agit d'un domaine controversé avec des implications éthiques, légales, et morales, car le tout implique une modification du contenu génétique humain. Ces inquiétudes doivent être adressées afin de pouvoir continuer à explorer les bienfaits de cette thérapie génétique. En poursuivant la recherche dans ce domaine, il serait possible de valider cette thérapie et optimiser ses bienfaits.

INTRODUCTION

Cancer is one of the most chronic and pressing health issues in the world today, causing over 8 million deaths per year, worldwide [1]. While existing surgical, radiation, and chemotherapeutical procedures have improved outcomes in some cancer types, many cancers still do not respond well to treatment [2]. Moreover, the overall prevalence and incidence rates of cancer are expected to continue to rise [2,3]. Consequently, there is a great impetus to explore novel approaches by which to treat different cancers in ways that current standard approaches cannot.

In the past decade, gene therapy has emerged as a possible avenue to produce innovative anti-cancer treatment strategies [4].

Gene therapy employs the targeted delivery of genetic material, or methods of genetic modification, to produce a positive therapeutic outcome by altering specific cells or tissues [5]. This approach could broaden the range of available treatment options through a number of techniques, including the insertion of foreign DNA into target cells to affect or restore protein expression, targeting viruses to abnormal cells for lysis and death, and even repairing deleterious genetic mutations [5]. Consequently,

Keywords: Gene therapy; Cancer; CRISPR; Immunotherapy; Gene transfer; Review

gene therapy may prove to be an essential tool in personalized medicine, as it can design treatments that are specific to a patient's genetic composition. Although many of these techniques are largely experimental, the field has made significant progress in the past decade, with some gene therapy options becoming commercially available. In the context of cancer research, recent developments such as cancer vaccines, oncolytic virotherapy, and gene transfer treatments are emerging as promising technologies to treat certain cancers with a better efficacy and safety profile than current standard treatment approaches [5].

This review aims to describe the current state of gene therapy research into applications for cancer, highlight the relative advantages and disadvantages these approaches have over existing therapeutic options, and address the limitations currently being faced by the field and the future directions needed to overcome them.

GENE TRANSFER TREATMENTS

Currently, one of the most widespread and well-established methods of gene therapy is the insertion of foreign genes into target cells through a number of different transfer methods. Collectively, these methods are referred to as "gene transfer treatments." One common approach is through viral vectors, usually belonging to a group of viruses called adenoviruses, which carry and release a therapeutic gene into a target tissue [5]. Adenoviruses are powerful tools in gene therapy due to the ease with which foreign genes can be inserted into their genomes, the relatively mild host immune response they provoke, their low rate of imprecise host genome integration (which decreases the possibility of unwanted mutations), and their lack of replicative ability (which prevents continuation of the lytic cycle and spread to other cells) [6].

Indeed, many adenoviral vectors have been developed for use in the treatment of cancer, and have generated a remarkable deal of excitement over their therapeutic impact. The drug Gendicine, which became the first commercially approved gene therapy treatment in the world in 2003, is a recombinant adenovirus that contains the gene for the tumour suppressor p53 [7]. Delivery of Gendicine to tumour cells allows for the overexpression of p53, and restoration of p53 activity in cells with dysfunctional copies of this gene [7]. Gendicine was a landmark in the history of anti-cancer gene therapy, particularly squamous cell carcinoma, as it stimulated the apoptosis of tumour cells, increased the expression of other tumour suppressor genes, decreased the prevalence of multi-drug resistance factors, and reduced vascular growth towards the cancerous tissues, all with fewer side effects than conventional chemotherapy and radiotherapy [7]. Other viral classes, such as retroviruses or adeno-associated viruses, are also used for gene therapy, each with their respective

strengths and efficiencies in different cell types. For example, the drug Rexin-G is a specially designed retrovirus that selectively integrates into the genome of pancreatic tumour cells [8]. It carries a modified gene encoding a construct that interferes with cyclin G1, thereby causing cell cycle arrest or death [8]. Rexin-G, which is currently in Phase III trials, has shown promise in the treatment of advanced pancreatic cancer as it increases mean survival time by almost 10 months compared to standard treatment [8,9].

Despite the promise that prospective gene transfer treatments may hold, several limitations and hurdles remain. In 2003, attempts to treat a rare disorder called X-linked severe combined immune-deficiency (SCID-X1) using a retrovirus-based agent unfortunately led to the development of T-cell leukemia in one patient due to integration of the virus within the patient's genome and subsequent genetic instability [10]. This highlighted the need to develop viral vectors that do not integrate into key regions of host DNA. Most current vector therapies are thus based on the safer adenoviruses or adeno-associated viruses, although these are often less effective at infecting a sufficient number of cells in target tissues to produce a clinically meaningful response. For example, these viruses often collect in the liver for unknown reasons, reducing their pharmacological efficiency and provoking a potentially harmful immune responses [5]. Furthermore, since the viruses do not replicate, additional viral loads must be injected periodically to sustain the expression of the therapeutic gene. Recurrent injections, however, trigger the development of an acquired immune response to the therapeutic viruses, which further reduces the efficacy of the treatment [11].

Currently, challenges relating to delivery methods are crucial deciding factors in the success of gene transfer treatments. To this end, viral vectors are becoming increasingly sophisticated, and non-viral means of transfer are emerging, such as the insertion of naked DNA directly into cells [12,13]. The ability to safely and precisely alter the function of tumour cells via gene transfer, in order to achieve the desired clinical outcome, will largely depend upon precise therapeutic delivery.

IMMUNOTHERAPY

Gene therapy can also be used to modify a patient's immune system in order to strengthen the response against cancer cells. Treatments that boost the immune system's ability to better target and destroy cancer cells are referred to as immunotherapy, and have been an aim of cancer treatment for more than a century [14]. However, the effectiveness of conventional immunotherapy is often limited by the ability of cancer cells to evade immune detection. As such, a number of different gene therapy techniques are being explored as methods to overcome this limitation [5].

One particularly interesting approach is that of cancer vaccines, which aim to cure or contain current cancerous growth by delivering material that trains the immune system to better recognize and attack cancer cells [4,15,16]. This is in contrast to prophylactic vaccines against bacteria or viruses, which are composed of molecules that mimic the infectious agent to help the body prevent future illness. The injected material of cancer vaccines is created by harvesting tumour cells from the patient's body and genetically modifying them through the addition of genes that produce antigenic and immunostimulatory proteins, such as those encoding for cytokines or other pro-inflammatory molecules [5]. This, in essence, serves to produce antigenic factors from the cellular debris, which are more potent than the endogenous tumour antigens. Upon injection, these modified tumour antigens increase the activity of antigen-presenting cells and cytotoxic T lymphocytes, thereby creating a stronger and more aggressive anti-tumour immune response [16]. Alternatively, a variation of this approach entails delivering the immunostimulatory and antigenic genes directly to the cancer cells in the body, which heightens immune recognition of these cells and leads to a more localized immune response [5].

A number of promising genetically modified cancer vaccines are currently being tested in clinical trials. A prominent example is GVAX, a vaccine which targets advanced pancreatic cancer and consists of tumour cells modified to express granulocyte-macrophage colony-stimulating factor (GM-CSF) [17,18]. The presence of GM-CSF secreted by the injected cells stimulates the release of cytokines at the injection site, which activates antigen-presenting cells, as well as CD4+ and CD8+ T cells, to better recognize the circulating tumour-associated antigens and strengthen the targeted immune response [18]. The vaccine is currently in Phase II trials and recent reports indicate that for treatment of pancreatic adenocarcinoma, patients administered with GVAX had significantly higher survival rates compared to those administered with standard chemotherapy [5]. Most of the side effects observed have been limited to minor injection site reactions or flu-like symptoms [16,18]. Moreover, another advantage of the vaccine is that it can be designed specifically for the patient using their tumour cells (called an autologous vaccine), thereby increasing its specificity for the patient's unique immunological environment.

While the previously described concept focused on modifying the cancer cells to induce a more potent immune response, another strategy is to modify the body's immune cells directly, in order to render them more aggressive towards tumour cells [19]. This is done by extracting and culturing lymphocytes from a patient's peripheral blood, and then genetically engineering them to overexpress potent cytokines like interleukin-2 (IL-2), or to produce T cell receptors (TCRs) that are specific for antigens on certain tumours [5,19]. Presently, T cells engineered to express TCRs against the NY-ESO-1 antigen have been successfully

employed in patients with metastatic melanoma and metastatic synovial cell sarcoma [20]. Trials have shown that 50–80% of patients demonstrate objectively better regression of the cancer compared to conventional chemotherapy, with no reported toxic side effects against other tissues [20]. One caveat of this technology, however, is that it is currently incredibly expensive. On average, genetic engineering of a patient's T cells is expected to cost between USD \$40,000 and \$75,000 [21].

In summary, immunotherapy is a promising therapeutic option that may be a source of future breakthroughs in personalized cancer treatment. The main hurdles, as it stands, are the immense cost and time needed to produce autologous vaccines and genetically engineered T cells. To this end, the replacement of autologous cells with allogenic cells (that is, derived from pre-existing cultured cell lines) is being investigated as a means to de-personalize and thereby streamline the process [5].

ONCOLYTIC VIROTHERAPY

A concept related to immunotherapy is oncolytic virotherapy, which employs genetically engineered viral particles to specifically target cancerous tumours [5,22]. These viruses do not replicate in healthy cells, and are therefore selective in their eradication of cancerous cells—an advantage over existing chemotherapy or radiation therapy [22,23]. While this is a relatively new and largely experimental area of research, several oncolytic viruses have performed very well in clinical trials, and some have been approved for market sale. Oncorine, an oncolytic virus used in nasopharyngeal cancer, is an adenovirus that has been engineered to lack the E1B protein, which is responsible for deactivating the p53 protein in the host cell [23]. The tumour suppressor p53 plays a crucial role in the host cell's ability to destroy the virus. Without viral E1B to deactivate the host's p53 defense, the host's normal cells will be able to clear the Oncorine infection. On the other hand, many cancerous cells have defective p53 genes (a main cause of neoplastic proliferation), and may therefore be infected by the Oncorine virus, resulting in toxicity/death [23]. Phase III trials of the drug have shown a response rate of 80% for head and neck tumours, which was double the rate in patients given standard chemotherapy [24]. Prior studies consistently demonstrated a good safety profile for Oncorine with only flu-like side effects [22,23].

A number of obstacles currently hinder the development of oncolytic virotherapies. First, the classes of viruses used to derive these therapies are fairly common in nature, meaning that many individuals will have been exposed at some point and therefore exhibit pre-existing immunity. Some possible solutions include the use of immunosuppressants to temporarily halt immune reactions, or carrier cells to deliver the viruses directly to the tumour [23]. Second, the rate of infection, replication, and death

Review

Table 1. Summary outlining the mechanisms, advantages, and limitations of each of class of gene therapy, as they pertain to cancer treatment.

Class	Mechanism	Advantages	Limitations
Gene Transfer Treatments	Insertion of foreign genes into target cells, mainly through viral based vectors [5]. Other non-viral delivery methods are being developed as well [12,13].	Can alter tumour cell function, restore apoptotic or tumour suppression pathways, and enable targeted disruption of specific types of cancer cells [5–8].	There are difficulties with achieving efficient transfer methods, host immune responses to many viral vectors, and concerns about safety due to increased risks of mutations [10,11].
Immunotherapy	Modifies cancer cells to produce antigenic and immunostimulatory molecules, or modifies immune cells to express cytokines and T cell receptors against specific tumour antigens [14–16].	Creates a more aggressive and targeted immune response toward tumours, Can create ‘cancer vaccines’ that are personalized for the patient’s tumour type and immunological environment [14–16].	Very time-intensive and expensive; some cost estimates can go up to USD \$75,000 [14–17,21].
Oncolytic Virotherapy	Produces genetically modified viruses which target and attack tumour cells preferentially over normal cells [22].	Allows for treatment directed specifically against cancer cells with minimal side effects. Also enables targeting of certain cancers that do not respond to well to standard therapy [23,24].	Host can develop immune response against the viruses. Also not efficacious against fast-growing tumours due to growth rates that exceeds viral replication rate [21,22].
Direct Gene Editing	Tools like CRISPR/Cas9 can be used to edit or replace sections of the patient’s genome in a highly accurate and practical manner [25,26].	Can remove latent cancer-causing viral infections such as HPV. Also, it is able to repair deleterious mutations, restore tumour suppression and apoptosis, and change alleles associated with cancer risk [25–30].	Current delivery methods of Cas9 constructs have difficulties with efficiency, and oncogenic viruses can develop resistance against the constructs [28]. There are also concerns over the ethics and effects of permanent genome modification [30–33].

of infected cancerous cells must be greater than the growth rate of the uninfected cancerous cells for the treatment to be able to effectively reduce tumour size. As such, this approach may not be suitable for very large or fast-growing tumours.

DIRECT GENE EDITING

The advent of CRISPR/Cas9 technology promises to revolutionize the field of gene therapy. This technique allows for precise modification of DNA sequences in an efficient and simple manner, which could serve many therapeutic purposes. CRISPR/Cas9 is one of the fastest-growing areas of gene therapy research, and has therefore generated a fair deal of excitement and controversy [25].

In the context of cancer research, the tool has shown remarkable success against viral infections linked to the development of cancer. For example, a CRISPR/Cas9 construct engineered to

specifically target and cleave the E6 oncogene of human papilloma virus (HPV) in cervical cancer cells showed a substantial reduction in HPV viral load and restoration of normal apoptotic genes [26]. Administration of a similar construct in HPV-infected mice with cervical cancer found a significant reduction in tumour size [27]. This is not without limitation however, as viruses have been shown to evolve resistance to CRISPR/Cas9 constructs [28]. Moreover, as with gene transfer treatments, inefficient therapeutic delivery presents a large barrier to eventual clinical implementation of this treatment [25]. Additionally, CRISPR/Cas9 technology could be used to repair deleterious mutations or replace sections of DNA with any desired sequence [25,29,30]. Correcting accumulated mutations within cancerous cells or prophylactically fixing alleles associated with increased cancer risk have both been suggested as ways to control the development of disease [25,29,30].

As the CRISPR/Cas9 tool rapidly develops, ethical, moral, and

legal questions inevitably ensue from the ability to modify the genetic content of human beings [30–33]. Current issues include the extent to which traits and parts of the genome should be allowed to be modified, the ethics of manufacturing “biologically superior” individuals, inequalities that would result from socio-economic access barriers, and the question of whether human embryos should be modified as a means to cure genetic diseases [30–33]. These approaches have also raised the concern of eugenics, provoking considerable controversy as well as opposition from several religious, philosophical, and legal bodies [32,33]. To this end, the developers of CRISPR/Cas9 have requested a ban on all attempts at human germline modification until society can have a discussion about its consequences [31].

CONCLUSION

Gene therapy is an exciting new technology that will generate novel medical procedures capable of targeting diseases like cancer in innovative ways. The development of gene transfer treatments, immunotherapy, oncolytic virotherapy, and direct gene editing are emerging as strong therapeutic applications (Table 1). They have demonstrated the ability to improve survival time and clinical benefit in many cancers that respond poorly to standard treatment options, while at the same time carrying fewer side effects than radiation and chemotherapy. However, many of these modalities are in experimental stages, and there are still some concerns over their safety, efficacy, and commercial viability. Moreover, the rapid development of gene editing technologies, such as CRISPR/Cas9, has led to controversy over the ethical, legal, and moral implications that human genome modification will have on society. These issues must therefore be addressed through prudent solutions and regulatory/legal frameworks before gene therapy and genome modification can become widely available for the treatment of human diseases [33].

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127(12):2893–2917.
2. Varmus H. The new era in cancer research. *Science*. 2006;312(5777):1162–1165.
3. Siegel R, Desantis C, Virgo K, et al. Cancer treatment and survivorship statistics, 2012. *CA Cancer J Clin*. 2013;62(4):220–241.
4. Fioretti D, Iurescia S, Fazio VM, Rinaldi M. DNA vaccines: developing new strategies against cancer. *J Biomed Biotechnol*. 2010;2010:174378.
5. Cross D, Burmester JK. Gene therapy for cancer treatment: past, present and future. *Clin Med Res*. 2006;4(3):218–227.
6. Hermonat P, Muzyczka N. Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells. *PNAS*. 1984;81(20):6466–6470.
7. Li Y, Li B, Li C, Li L. Key points of basic theories and clinical practice in rAd-p53 (Gendicine) gene therapy for solid malignant tumors. *Expert Opin Biol Ther*. 2015;15(3):437–454.
8. Gordon E, Hall F. REXIN-G, a targeted genetic medicine for cancer. *Expert Opin Biol Ther*. 2010;10(5):819–832.
9. Chawla SP, Chua VS, Fernandez L, et al. Advanced phase I/II studies of targeted gene delivery in vivo: intravenous REXIN-G for gemcitabine-resistant metastatic pancreatic cancer. *Mol Ther Nature Publishing Group*. 2010;18(2):435–441.
10. Fischer A, Hachein-bey-abina S, Cavazzana-calvo M. Gene therapy for primary adaptive immune deficiencies. *J Allergy Clin Immunol*. 2011;127(6):1356–1359.
11. Ohlfest JR, Demorest ZL, Motooka Y, et al. Combinatorial antiangiogenic gene therapy by nonviral gene transfer using the Sleeping Beauty transposon causes tumor regression and improves survival in mice bearing intracranial human glioblastoma. *Mol Ther*. 2005;12(5):778–788.
12. Patil S, Rhodes D, Burgess D. DNA-based therapeutics and DNA delivery systems: a comprehensive review. *AAPS J*. 2005;7(1):E61–E77.
13. Zinn E, Pacouret S, Khaychuk V, et al. In silico reconstruction of the viral evolutionary lineage yields a potent gene therapy vector. *Cell Rep*. 2015;12(6):1056–1068.
14. Armstrong AC, Eaton D, Ewing JC. Cellular immunotherapy for cancer. *BMJ*. 2001;323(7324):1289–1293.
15. Nawrocki S, Wysocki P, Mackiewicz A. Genetically modified tumour vaccines: an obstacle race to break host tolerance to cancer. *Expert Opin Biol Ther*. 2001;1(2):193–204.
16. Hege K, Jooss K, Pardoll D. GM-CSF gene-modified cancer cell immunotherapies: of mice and men. *Int Rev Immunol*. 2006;25(5-6):321–352.
17. Eager R, Nemunaitis J. GM-CSF gene-transduced tumor vaccines. *Mol Ther*. 2005;12(1):18–27.
18. Lipson E, Sharfman W, Chen S, McMiller T, Pritchard T, Salas J. Safety and immunologic correlates of Melanoma GVAX, a GM-CSF secreting allogeneic melanoma cell vaccine administered in the adjuvant setting. *J Transl Med*. 2015;13:214–238.
19. Rosenberg S. Raising the bar: the curative potential of human cancer immunotherapy. *Sci Transl Med*. 2012;4(127):127ps8.
20. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol*. 2011;29(7):917–924.
21. Sharpe M, Mount, N. Genetically modified T cells in cancer therapy: opportunities and challenges. *Dis Model Mech*. 2015;8(4):337–350.
22. Russell S, Peng K, Bell J. Oncolytic virotherapy. *Nat Biotechnol*. 2012;30(7):658–670.
23. Guo Z, Thorne S, Bartlett D. Oncolytic virotherapy: molecular targets in tumor-selective replication and carrier cell-mediated delivery of oncolytic viruses. *Biochim Biophys Acta*. 2008;1785(2):217–231.
24. Garber K. China approves world’s first oncolytic virus therapy for cancer treatment. *J Natl Cancer Inst*. 2006;98(5):298–300.
25. Barrangou R, May AP. Unraveling the potential of CRISPR/Cas9 for gene therapy. *Expert Opin Biol Ther*. 2014;15(3):1–4.
26. Yu L, Wang X, Zhu D, et al. Disruption of human papillomavirus 16 E6 gene by clustered regularly interspaced short palindromic repeat/Cas system in human cervical cancer cells. *Oncotargets Ther*. 2014;8:37–44.
27. Platt R, Chen S, Zhou Y. CRISPR/Cas9 knockin mice for genome editing and cancer modeling. *Cell*. 2014;159(2):440–455.
28. Wang Z, Pan Q, Gendron P, et al. CRISPR/Cas9-derived mutations both inhibit HIV-1 replication and accelerate viral escape. *Cell Rep*. 2016;15(3):1–9.
29. Doudna J, Charpentier E. The new frontier of genome engineering with CRISPR/Cas9. *Science*. 2014;346(6213):1258096.
30. Lokody I. Genetic therapies: correcting genetic defects with CRISPR-Cas9. *Nat Rev Genet*. 2014;15(2):63.
31. Baltimore D, Berg P, Botchan M, et al. A prudent path forward for genomic engineering and germline gene modification. *Science*. 2015;348(6230):36–38.
32. Lanphier E, Urnov F, Haecker S, Werner M, Smolenski J. Don’t edit the human germ line. *Nature*. 2015;519(7544):410–411.
33. Ishii T. Potential impact of human mitochondrial replacement on global policy regarding germline gene modification. *Reprod Biomed Online*. 2014;29(2):150–155.