1 Introduction

Gene therapy is the treatment of diseases via the modification of DNA in appropriate target cells. It has recently become a clinical reality after nearly 30 years of laboratory research. The first gene therapy clinical trial was launched in September 1990 at the National Institute of Health Clinical Center to treat adenosine deaminase deficiency, a genetic disease that makes patients vulnerable to infections\(^1\). As of June 2012, over 1 800 gene therapy clinical trials have been completed, are ongoing or have been approved worldwide, of which 1 448 trials are in phase I or I/II, 323 are in phase II or II/III and 67 are in phase III.\(^2\) A recombinant human adenovirus-p53 injection (trademarked as Gendicine) was approved by the State Food and Drug Administration (SFDA) of China in October 2003 for the treatment of head and neck squamous cell carcinoma.

Review

The roles of traditional Chinese medicine in gene therapy

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ABSTRACT: The field of gene therapy has been increasingly studied in the last four decades, and its clinical application has become a reality in the last 15 years. Traditional Chinese medicine (TCM), an important component of complementary and alternative medicine, has evolved over thousands of years with its own unique system of theories, diagnostics and therapies. TCM is well-known for its various roles in preventing and treating infectious and chronic diseases, and its usage in other modern clinical practice. However, whether TCM can be applied alongside gene therapy is a topic that has not been systematically examined. Here we provide an overview of TCM theories in relation to gene therapy. We believe that TCM theories are congruent with some principles of gene therapy. TCM-derived drugs may also act as gene therapy vehicles, therapeutic genes, synergistic therapeutic treatments, and as co-administrated drugs to reduce side effects. We also discuss in this review some possible approaches to combine TCM and gene therapy.

KEYWORDS: gene therapy; medicine, Chinese traditional; reviews

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making it the world’s first gene therapy product approved by a governmental agency [15]. Currently, Gendicine is being extensively studied in other clinical trials for various cancers [16-18]. More recently, in November 2012, the recombinant adeno-associated viral (rAAV) vector Glybera, used to treat lipoprotein lipase deficiency (LPLD), received marketing authorization from the European Commission, becoming the first gene therapy product in the Western world [6,7]. All these clinical breakthroughs make gene therapy one of the most innovative areas in current biomedical study.

Traditional Chinese medicine (TCM), an important component of complementary and alternative medicine, has evolved over thousands of years with a standardized system of theories, diagnostics and therapies. In China, TCM practitioners are using a variety of techniques in an effort to promote public health and to treat diseases and recently these techniques have drawn much attention in the Western world [8-10]. During the past half century, the role of TCM in preventing and treating infectious diseases such as hepatitis [10], acquired immune deficiency syndrome [11], and chronic diseases such as cancer [12], hypertension [13], diabetes [14] and cardiovascular diseases [15] has become widely accepted. However, despite the encouraging research, the role that TCM may play in gene therapy, a relatively new biomedical practice, has not yet been systematically discussed. Here we provide an overview of the TCM theories in relation with gene expression and then discuss example approaches to combine TCM and gene therapy.

2 Theoretical basis of TCM in relation with gene expression

TCM is based on the guiding principles of viewing the human body as a whole, and syndrome differentiation of the individual case. The Yellow Emperor’s Internal Classics, or Huangdi Neijing, was written over 2,000 years ago and is the oldest complete Chinese medical text. It stated that a person’s health is related in part to what he receives while in the womb, which is inherited from the parents, stored in the kidneys, and called kidney essence. Modern TCM theories correlate the kidney essence with genetic material, which is also the basis of human health. The constitution theory of TCM indicates that people all possess his or her unique constitution, which includes different amounts of kidney essence. This can be seen in an individual’s unique onset of diseases, clinical manifestations and the subsequent prognoses. Similarly, modern medicine also holds that an individual’s susceptibility to pathogenic factors, diversity of clinical manifestations, and responses to drugs are closely related to his or her genetic factors and gene polymorphism. More than 20 years ago, researchers found that human leukocyte antigen, which is closely related with human diseases, has some characteristics such as polymorphism and the phenomenon of linkage disequilibrium. These characteristics have much in common with the constitution theory of TCM [16].

In the area of treatment, TCM doctors use observation, auscultation and olfaction, interrogation, and palpation for diagnosis and summarization of the patient’s individual syndrome, and the treatment is based on this TCM syndrome. This is called syndrome differentiation, a main clinical process in TCM. Under this guide, patients with the same disease may have various TCM syndromes, which is shaped by each patient’s condition, such as kidney essence (or genetic background) and living environment. The treatment of illness in TCM clinical practice is primarily guided by the patient’s TCM syndrome. Interestingly, modern biomedical research suggests that each TCM syndrome may have a specific gene expression profile. For instance, Weng et al. [17] reported differential gene expression in peripheral blood mononuclear cells isolated from hepatocellular carcinoma (HCC) patients, either with or without liver-kidney yin deficiency syndrome, using a gene chip technique. Analysis of the data showed that both the mRNA and protein expression levels of SEC62, cyclin B1 and baculoviral IAP repeat-containing 3 (BIRC3) are significantly lower in HCC patients with syndrome of liver-kidney yin deficiency. Meanwhile, Wei et al. [18,19] reported that the expression of at least 79 genes was disrupted in patients with syndrome of kidney yin deficiency, compared to normal populations. The difference was even more striking between patients with kidney yin deficiency syndrome versus those with kidney yang deficiency syndrome, with more than 145 differentially expressed genes identified. All these differences may be targets for gene therapy in specific diseases. Thus, we believe that there are some common medical theory bases between the individual therapy guided by the holistic concept of TCM and the experimental research based on gene therapy.

3 TCM agents as gene therapy vehicles

To date, a variety of physical, chemical and biomedical methods have been developed to introduce exogenous DNA sequences into eukaryotic cells, including the use of viral and non-viral vectors. Each method has its own pros and cons: viral vectors based on retroviruses and adenoviruses have already been employed in a number of clinical trials but have caused serious side effects [20-24], rAAV vector is not associated with any known disease. However, the inability to transfer large size genes significantly limits its usage [25]. Furthermore, all viral vehicles are immunogenic, leading to inflammation, followed by their removal by the host immune system, as well as the inability to readminister the same viral vector. Non-viral vectors, including synthetic liposomes [26], cationic lipids [27], polymer [28] and DNA nanoparticles [29],
on the other hand, provide advantages including no limitation to the size of transgenic DNA, lower immunogenecity, and ease of preparation\[30\]. However, their efficiency, specificity, cost-effectiveness and potential cytotoxicity are generally less well-understood.

The first attempt to use TCM herbs as vehicles for the purpose of gene therapy by oral delivery was reported and discussed by Dr. Zheng and his colleagues\[31\]. They constructed transgenic tobacco through the infection of recombinant tobacco mosaic viral (rTMV) vectors carrying a chloramphenicol acetyltransferase (CAT) gene, a bacterial enzyme that detoxifies the antibiotic chloramphenicol. The transgenic tobacco produced large amount of rTMV-CAT DNA and its corresponding mRNA as well as proteins. Interestingly, oral delivery of the decoction of the transgenic tobacco in rats resulted in detectable CAT activity in rat jejunal mucosa one week post-administration. Although other explanations may exist\[32\], one of the potential mechanisms involves the delivery and expression of exogenous nucleic acids in rat gastrointestinal mucosa. Theoretically, nucleic acids in herbs and animal cells will be denatured during the process of decoction, followed by further denaturation by gastric acid after oral delivery, annealing by success entericus and most importantly, emulsion to form lipid/DNA complex by bile acid, and finally be delivered into eukaryotic cells as liposomes. However, several questions still need to be fully studied such as the delivery efficiency, size limitation and the potential immune response. In addition to the decoction, efforts were also made in pursuing purified molecules from TCM herbs. It was reported that cationic polysaccharide isolated and prepared from Bletilla striata has the ability to incorporate and protect plasmid DNA from DNase digestion\[33\]. Moreover, the polysaccharide/DNA complex can be successfully transfected into cultured cell lines in vitro and into hepatocytes in vivo when administered through the hepatic artery injection. Another promising alternative for gene delivery vectors is the development of flexible nanotubes from natural polymers. Carbon nanotubes have been extensively explored as a nonviral delivery system\[34,35\] and more recently, we and others synthesized nanotubes based on cell wall polymer lignin isolated from aromatic plants such as Sorghum bicolor and sugar cane Saccharum spp\[36,37\]. Cytotoxicity studies with human HeLa cells showed that concentrations of up to 90 mg/mL are tolerated, which is a 10-fold higher concentration than that observed for carbon nanotubes. Confocal microscopy imaging revealed that all lignin nanotube formulations enter HeLa cells without auxiliary agents, and those made from NaOH-lignin have the ability to penetrate the cell nucleus. We further showed that DNA can be absorbed to the lignin nanotubes. Consequently, exposure of HeLa cells to the lignin nanotubes coated with DNA encoding the green fluorescent protein (GFP) leads to transfection and expression of GFP in the cells. These combined features make lignin nanotubes attractive as delivery vehicles of DNA and studies to reveal their efficacy and potential cytotoxicity in mice in vivo are currently undergoing in our laboratory.

Some TCM peptides have the activity to lyse cell membranes, which can be used as vehicles to deliver exogenous DNA. Bee venom, as a commonly used TCM drug, has antibacterial, antiviral, and anti-inflammatory effects\[38\]. Melittin is the principal toxic component in the venom of the honey bee Apis mellifera and is a cationic membrane lytic peptide\[39\]. Dr. Ogris and his colleagues\[40\] covalently linked melittin, a 26 amino-acid peptide to the cationic polymer poly-ethylenimine (PEI), followed by conjugation with plasmid DNA. Compared with unmodified PEI, the transfection activity was strongly increased within a broad range of cell lines and it is evident that enhanced membrane lytic activity is one of the potential mechanisms. Next, it is of interest to determine whether this technology can be applied for PEI-mediated delivery of therapeutics in vivo\[41\]. Recently, Zhang et al\[42\] constructed novel gene vectors by coupling the stearyl moiety to the N-terminus of the melittin and reported that the transfection efficiency can be increased more than 10-fold compared with unmodified stearyl. Again, the efficiency of this novel system needs to be determined in vivo.

The delivery mechanism for TCM drugs has made a variety of TCM derivatives into liposomes, including ginsenosides, tetrandrine, Panax notoginseng saponins, and taxol\[43\]. However, modification of liposomes by conjugating to small molecules isolated from TCM botanicals has been less well studied. Some studies have provided encouraging results. For instance, Mao et al\[44\] synthesized 3-succinic acid-30-stearyl alcohol glyceryl-18 acid ester, which had specific affinity to the liver, and prepared cantharidin polyphase liposome modified with this molecule through the method of ethanol injection. The results indicated that the addition of the molecule did not affect the physical and chemical properties of the liposome, and the hepatic concentration of the modified polyphase liposome was significantly higher than those of the unmodified liposome, after intravenous injection in mice. Theoretically, a liposome with different glycosyls combining on the surface can get different distributions in vivo, and these surface-modified liposomes are promising vectors for the purpose of gene therapy.

4 TCM agents as therapeutic genes

Another potential area of use for TCM drug therapy involves various proteins and peptides from herbs and animals that may serve as promising suicide genes to treat malignant diseases such as cancer. Melittin, as mentioned above, is a 26 amino-acid peptide
and exemplifies a large class of membrane-active peptides that manifest membrane-disrupting activity, especially when incorporated with other delivery systems\[45\]. Various cancer cells, including renal, lung, liver, prostate, bladder, mammary and leukemia cells, can be targets of bee venom\[38\]. Li et al\[46\] constructed a recombinant adeno-viral vector carrying the melittin-encoding gene driven by a liver cancer-specific promoter, a-fetoprotein promoter (AFPp) and showed that it inhibited the proliferation and induced apoptosis of the liver cancer cell lines. Trichosanthin (TCS) is the main bioactive protein component isolated from the root tubers of *Trichosanthes kirilowii*, which has been used as an abortifacient for over one thousand years in China\[47\]. Its enzymatic activity was recognized as type I ribosome-inactivating protein\[48\], which is responsible for its antitumor effect through systemic administration of the protein\[49\]. Although the nucleotide sequence of TCS gene was determined more than 20 years ago\[50\], only in recent years has the delivery of TCS-encoding gene into cells been pursued. Peng et al\[51\] constructed a recombinant TCS-expressing plasmid and demonstrated that the expression of TCS in human cervical cancer cells, Caski, significantly inhibits the cell growth *in vitro*. Although the underlying mechanism still needs to be fully studied, our team is attempting to achieve suppression of tumorigenesis in a human liver cancer xenograft mouse model through systemic administration of optimized rAAV3 vectors carrying TCS gene driven by the AFPp\[52-54\]. Another example is the study of an analgesic-antitumor peptide (AGAP) from *Buthus martensii* Karsch. Again, it was cloned into an expressing plasmid under the driven of AFPp by Jin et al\[55\] and the results indicated that the expression of AGAP in HepG2 cells significantly inhibits their growth.

Recently, Dr. Zhang’s group reported an interesting finding that exogenous plant microRNAs (miRNAs) are present in the sera and tissues of various animals through food intake\[56\]. Previously, the same group has demonstrated that some miRNAs, which are abundant in rice, are also the most highly enriched exogenous miRNAs in the sera of Chinese subjects\[57\]. Even more strikingly, it is evident that these circulating plant miRNAs have the ability to regulate the expression of target genes in host mammals. Although there is still debate regarding the interpretation of the data\[58,59\], it may provide the basis for a new mechanism for how TCM herbs interact with the human body.

### 5 TCM agents as enhancer for transgene expression

Transgene expression is one of the biggest concerns in the process of gene therapy. The process of increasing transgene expression at reduced vector dose remains a critical challenge for the successful use of most, if not all, vectors in clinical applications. For example, vectors based on rAAV have been successfully utilized in a recent gene therapy clinical trial for hemophilia B\[60\]. The optimization of the vectors in this trial included a switch of the rAAV capsid from serotype 2 to serotype 8, a self-complementary rAAV genome instead of a single-stranded one, which bypasses the rate-limiting viral second-strand DNA synthesis\[61,62\] and a codon-optimized factor IX encoding gene\[63\]. However, large vector doses (2 × 10¹² viral genomes/kg) are still needed, which elicit CD8⁺ T cell responses to viral capsid at those doses required to achieve FIX levels of 5%-10% of the normal. To address this issue, numerous chemical and physical methods have been developed to enhance gene therapy efficiency. For instance, a number of chemotherapeutic agents have been used to induce cell stress and to enhance rAAV transduction including proteasome inhibitors\[64,65\] such as MG-132, calpain inhibitor I, and bortezomib; DNA synthesis inhibitors\[66\] such as hydroxyurea and aphidicolin; and topoisomerase inhibitors\[67\] such as etoposide and camptothecin. TCM drugs may also play important roles in enhancing gene therapy efficiency.

More recent data have suggested that the bioactive monomeric compounds extracted from TCM herbs can be utilized together with viral vector-based gene therapy to enhance its transduction efficiency. Zhang et al\[68\] reported that celastrol, a chemical monomer isolated from the root bark of *Tripterygium wilfordii*, has the ability to significantly enhance rAAV1 serotype vector-mediated transgene expression in the adipose tissues of mice. The mechanism of the enhanced activity involves inhibition of cellular proteasome activity, which has been revealed to play an important role in rAAV transduction\[65\]. Later, Wang et al\[69\] showed that a methylated isomer of celastrol isolated from *Celastrus hypoleucus*, named pristimerin, has a relatively lower half maximal inhibitory concentration (IC₅₀) to inhibit cellular proteasome activity, which makes it more efficient in facilitating both rAAV2 and rAAV8 transduction efficiency *in vitro* and *in vivo*. Meanwhile, Dr. Samulski’s group demonstrated that a compound of arsenic trioxide, an US FDA approved chemotherapeutic agent, plus a well-known TCM drug called Pishuang, produces reactive oxygen species (ROS) in the cells and stabilizes the accumulations of rAAV virions at the perinuclear region. Thus the compound leads to an increase in the intracellular vector genome copy number and the subsequent transgene expression both *in vitro* and *in vivo*\[70\]. Our research is testing another ROS generator, bufalin, a cardiotonic steroid originally isolated from the Chinese toad venom, and the transduction efficiency of recombinant adenoviral vectors (unpublished data). All these efforts suggest the potential beneficial use of TCM-derived proteasome inhibitors and ROS generators in future gene therapy with viral vectors.
6 TCM agents as a synergistic therapeutic treatment

TCM has evolved over thousands of years with specific systems of theories, diagnostics and therapies for various conditions. Gene therapy, in comparison, is a relatively new field experimentally used in a handful of diseases: mainly inherited genetic disease such as LPLD and hemophilia; and malignant, non-cured diseases such as cancer. Still, numerous efforts are being made to combine elements of TCM treatments with gene therapy treatments, to reach synergistic therapeutic effects.

Delisheng (DLS), a Chinese herb-derived drug that is composed of Renshen (Panax ginseng), Huangqi (Astragalus propinquus), secretions of Rhabdias bufonisand cantharidium from Lytta vesicatoria, is commonly used for treating HCC and other cancers[71,72]. In 2006, a synergistic antitumor effect was observed when DLS was used together with Gendicine (a recombinant human adenovirus-p53 injection) in a liver cancer-bearing mouse model[73]. Promising data also came from clinical trials of a Chinese herb-derived drug, Aidi Injection, used for tumor treatment. Its main components include Banmao (Lytta vesicatoria), Ciwuja (Acanthopanax senticosus), Huangqi and Renshen[74]. Shi et al[75] suggested that patients with primary HCC may have significantly enhanced therapeutic effects when treated with both Gendicine and Aidi Injection.

In addition to antitumor drugs derived from Chinese herbal medicine, TCM agents that have immune-modulating functions may also be used together with gene therapy to treat cancer[76]. Liuwei Dihuang (LWDH) is a classic Chinese medical formula, which is comprised of Dihuang (root of Rehmannia glutinosa), Shanzhuyu (fruit of Cornus officinalis), Danpi (root bark of Paecania suffruticosa), Shanyao (rhizome of Alisma plantago-aquatica), Fuling (Dioscorea opposita) and Rehmannia glutinosa), Shanzhuyu (fruit of Paeonia suffruticosa), Cornus officinalis), which is known to have antitumor[85] and immune-modulating abilities[86], can be combined with plasmid-mediated anti-signal transducer and activator of transcription 3 siRNA delivery to treat malignant cells both in vitro and in vivo; and (5) Li et al[89] reported that diallyl trisulfide, isolated from Allium sativum reported that periplocin isolated from Wujiapi (Cortex Periplocae), which is known to have antitumor[88] and immune-modulating abilities[89], can be combined with plasmid-mediated anti-signal transducer and activator of transcription 3 siRNA delivery to treat malignant cells both in vitro and in vivo; and (5) Li et al[89] reported that diallyl trisulfide, isolated from Allium sativum, can be combined with gene therapy treatment against proliferation of rabbit lens epithelial cell through rAAV-mediated overexpression of HSV-TK/GCV genes. Additional well-designed laboratory research is needed to move these promising preliminary observations toward potential clinical application.

7 TCM agents as co-administrated drugs to reduce side effects

To date, most, if not all, gene therapy clinical trials have had some level of side effects, especially when high doses of vehicles were administrated. Theoretically, all virus-based gene therapy vectors will elicit a host immune
response upon systemic administration. On the other hand, some TCM drugs, especially in the class known as tonifying herbs, have the ability to modulate the host immune system and significantly reduce the negative effects from physical or chemical treatment. For example, much evidence shows that TCM plays a significant role in reducing adverse effects induced by chemotherapy in tumor treatment⁹⁰. Although few studies have been performed to reveal the roles of TCM drugs in reducing side effects resulting from gene therapy treatment, Wang et al⁹⁰ have observed TCM-derived agents to help significantly reduce persisting viral vectors in the circulating system.

8 Summary

To summarize, the combination of TCM agents with gene therapy, which is one of the most “modern Western” therapies, is a feasible concept. TCM-derived drugs can be used as gene therapy vehicles; they can be delivered into cells as therapeutic genes; and they can be combined with gene therapy to treat diseases. However, for most cases, extensive laboratory studies are needed before future clinical trials: (1) efficacy and potential cytotoxicity need to be determined; (2) underlying mechanisms need to be fully understood; (3) and most importantly, TCM theory also needs to evolve to support its modern clinical application. In addition, gene therapy is still in its infancy, with only two products world-wide approved for wide usage. It is reasonable to expect that increasing efforts will be made to combine these two previously-unrelated methods to enhance therapeutic outcomes. We believe that it will be a growing and challenging field in modern medicine.

9 Competing interests

The authors declare that they have no competing interests.

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