

The Application of Nanoparticles for Gene Vector in Tumor Gene Therapy

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ABSTRACT

Development of safe and effective carriers is one of the major difficulties for gene therapy. Commonly used carriers for gene delivery can be classified into two categories: viral carriers and non-viral carriers. Nanoparticles gene carrier of various different kinds of materials, which belong to non-viral carriers, are paid attentions widely for its advantages such as low toxicity and immunogenicity, good biocompatibility, easy to produce, and a promising perspective for application in tumor gene therapy. The nanoparticles have a high stability in organisms and cells, which was difficult to be degraded. Nanoparticles can improve transfection efficiency. As a nonviral gene vector, the application of nanoparticles in gene therapy became research focus. In this article, we mainly reviewed the recent studies of the characteristics of nanoparticles, characteristics and transport mechanism of nanoparticles as gene vector, the progress on nanoparticles as gene vector in tumor gene therapy.

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Key words: Nanoparticles; Gene vectors; Transfection efficiency; Tumor

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INTRODUCTION

Nowadays the morbidity and mortality of tumors are increasing year by year and seriously threaten the health of human. Traditional surgery can resect the lesion but the residue cancer cells can be the origins of the recurrence and metastasis of malignant tumors. And chemotherapy and radiotherapy are greatly restricted in clinical use for their serious side effects and multidrug resistance^[1]. In recent years with the development of molecular biology the application of gene therapy can completely clear the lesion without serious toxic and side effects and these reasons make it become the development trend in the treatment of tumors.

Gene therapy is a major advance in modern medicine and it is also the hotspot of tumor therapy research. The key to successful realization of gene therapy is the gene delivery mode, so it's important to choose a suitable gene delivery system that can make the plasmid DNA distributed in specific nucleus and inserted into specific DNA site. Scholars have done large numbers of studies on tumor gene therapy over the years, but influenced by many factors gene therapy effect is not ideal till the present time. It is still a very urgent and difficult problem about how to improve the efficiency of gene transfer, efficiently, targeted and harmlessly deliver the gene into a target site to achieve its therapeutic effect on the molecular level^[2].

TYPES, MATERIALS AND MECHANISM OF NANOPARTICLE GENE VECTOR

At present there are two existent gene vectors defined as viral vectors and non-viral vectors^[3-4]. Although retrovirus and adenovirus have high transfection efficiencies as representatives of the viral vectors, there were existed problems such as complex preparation, immunogenicity, serious security hidden danger and can't be repeatedly used in vivo so they can't become the satisfactory gene vectors^[5-6]. And the non-viral vectors use the synthetic nonbiological materials to bring the nucleic acids into the cells, have some good properties like non-toxic, easy to design and low immunologic reaction while the transformation efficiency is low^[7-8].

Nano-gene vector is usually some kind of nanocapsule or nanoparticles that prepared from biocompatible material, it can form nano-carrier gene complex by encapsulated or absorbed nucleic acid molecules like exogenous DNA. The particle size of the nanoparticles carrier is usually 10-100 NM and chemistry activity from the huge specific surface area makes it has outstanding

adsorption, concentration and protection ability, this is one of the main causes for nano-gene carrier exerts its adsorption, transport function on exogenous genes^[9-10]. The nano gene carrier materials are divided into organic and inorganic materials two. Organic materials are mainly high molecular polymer including dendritic polymers, cationic liposome, PLGA and Chitosan, inorganic nano material stability, with good dispersion behavior, easy preparation, easy to control the particle size, large loading quantity, no immunogenicity, low cytotoxicity and easy to achieve targeted delivery with coupling specific molecules on the surface to improve transaction efficiency. Inorganic materials mainly include silica, iron oxide and gold nanoparticles^[11-15]. The advantages and disadvantages of various gene nanoparticle carrier as shown in table 1.

Table 1 Type, advantages and disadvantages of gene carrier.

Carrier type	Advantages	Disadvantages	Representative materials
Viral vector	High transfection efficiency	The preparation of complex, The immunogenicity Unable applied repeatedly	Retroviruses; adenovirus
Biological particles nanometer carrier	Biological affinity Good targeted	Low transfection efficiency Prone to immune response	Liposomes; protein
Polymer nanoparticle Carrier	Good biodegradation Good biocompatibility	Occasional cytotoxicity Low transfection efficiency Need to surface modification	PLGA; Chitosan; Gelatin
Magnetic metal nanoparticle carrier	The simply preparation Specific binding Small toxic and side effects Largely used	Reunion phenomenon Need surface modification Less load	Magnetic iron oxide; Nano gold

Nano gene vectors have the ability to combine and concentrate DNA and RNA then efficiently import them into various cells. Then enter the cytoplasm through the interaction between the surface cation and the glycoproteins and phospholipid in the membrane with negative charge, the number of cations and their gene transfer rate is positively correlated^[16]. The nanoparticles can combine with DNA to form complexes and nanoparticles coupling with DNA molecules through electrostatic adsorption or their chemical bond. This combination can effectively protect the exogenous DNA that combined with nano particles from various enzyme digestions, and improve the transfection efficiency^[17]. Endocytosis is the main mechanism of the complex entered into the cells. Meanwhile, coupling specific targeted molecules such as specific antibodies or monoclonal antibodies on the surface of the nanoparticles and binding to specific receptors by targeting molecular on a cell membrane, under the action of the cell uptake the particles the gene into the cell and so that the gene transaction can be safe and effective targeted. Nanoparticles can mediate the DNA integrate into the nuclear genome and obtain the stable expression of exogenous gene.

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THE APPLICATION OF POLYMER MATERIALS NANOMETER GENE VECTOR IN TUMOR TREATMENT

Baker, University of Michigan Medical School immunologist and Nanomedicine technology authority, developed a nanoscale dendritic polymer, after injecting the carrying DNA dendritic polymer into the tissue its size is fit to enter the cell by endocytosis and then the DNA molecules released and into the nucleus to achieve gene integration^[18-19]. Nian-Feng Sun *et al.*^[20-23] prepared the cationic nano-liposome and tested its phenotype then using it as a gene carrier to study the mechanism of the colon carcinoma,

indicating that it has advantage than the viral vector in cytotoxicity but the gene transfection efficiency is still lower. Kaul *et al* using the PCMV-beta gene polyethylene gel nanoparticles and PCMV-beta gene were transfected into Lewis lung cancer cells respectively with nearly 100% encapsulation efficiency and 200 nm average diameter, high efficient transfection and expression was in control group, and *in vivo* studies on female C57BL/6J rats transplanted Lewis lung cancer found that both vein or intratumoral injection all have significant differences in gene expression between PCMV-beta gene polyethylene gel nanoparticles and the control group, and this provide new ideas for tumor gene therapy^[24].

THE APPLICATION OF MAGNETIC NANOMETER GENE VECTOR IN TUMOR TREATMENT

Now with the development of bionanotechnology the applications of nano-magnetic particles and gold nanoparticles in biomedical field have increasingly become one of the hot spots and they have obvious advantages in cell targeting transfection efficiency especially^[25]. Nano-magnetic particles' application in gene transfection is a new emerging multi-subjects research field, its proposition and development is built on the basis of nano-magnetic particles' materials science research results and non-viral gene transfection biological research^[26]. The study showed that during the process of magnetofection the magnetic field can't pull the magnetic particles into the cells directly and also did not change the mechanism of gene complexes' endocytosis, but accelerated gene complexes' sedimentation to the cell surface so as to promote the phagocytosis of gene complexes greatly, thereby enhanced gene transfection^[27]. Through the force of external magnetic field in the process of magnetofection the magnetic gene particle's sedimentation to the cell surface can be accelerated, thus promoted the phagocytosis of gene complexes and achieved the enhancement of gene transfection. The results of experiment showed that magnetofection is a kind of high efficiency, low toxicity and widely

applied gene transfection method, and a plasmid DNA harboring target genes or a synthesized small interference RNAs (siRNA) all can be efficiently transfected into cells through it in a short time, thus overcomes the defect of the lack of targeted without external magnetic field in gene transfection and makes more DNA enriched to the surface of a target cell, these all let it become the best choice for local targeted gene therapy in vivo^[28-30].

THE APPLICATION OF GOLD NANOMETER GENE VECTOR IN TUMOR TREATMENT

Gold nanoparticle is a kind of new material with many biological functions, mainly metabolized via urine and has no apparent cytotoxicity. The study showed that the unmodified gold nanoparticles had poor ability to carry DNA but the gene transfection efficiency can be improved after the superficial modification. Due to the gold nanoparticles have characteristics such as good stability, high surface activity, strong ability to carry genes and special biological affinity so they have been applied to some areas' exploring research like DNA testing, rapid immunodiagnosis, biosensor and targeted drug carrier in the world at present^[31-35].

With the development of Bio-nanotechnology more and more researches showed that superficial modified gold nanoparticles can greatly improve the gene transfection efficiency and have incomparable advantages in carrying genes for gene therapy^[36]. Now in using nano material as an assembly system structure unit, the latest research hotspot is the assemblies of multi-functional materials for that gold nanoparticles and nano-magnetic particles have excellent genes carrying capacity and targeting functions. Some studies have reported that the polyethylenimine (PEI) on the surface of nano-magnetic particles can prepare gold nanoparticles by reduction of chloroauric acid so goldmag bifunctional nanocomposite system is expected to become a good gene vector material^[37].

CONCLUSION

As a new drug and gene carrier nano-gene vector has some characteristics such as controlled released, target directed and higher bioavailability, and shows very good efficacy to some tumor animal model. But most of the current studies are still in vitro and in an animal experimental stage that need to be further researched and do some human experiments to improve targeted therapy efficacy and the rate of early cancer diagnosis. In order to improve the transfection efficiency of nano particles /DNA, some substances holding a positive charge such as poly-L-lysine and PEG can be modified on the surface of the nanoparticles, this will be more conducive to the formation of nano-particles / DNA complexes. As gene vector nanoparticle targeted relatively poor in gene therapy, but with coupling specific targeted molecules on the surface and through targeted molecules binding to specific surface receptors can achieve targeted and efficient gene transportation. Although the tumor-targeting gene therapy research has a long way to go, but I believe that tumor targeted therapy has a bright future under the efforts of numerous researchers.

FUTURE PERSPECTIVES

Currently, Gene therapy is essentially a question of purpose gene, vectors and transfection, gene delivery way is the key to the success of gene therapy. Ideal gene delivery carrier will be at the same time high transfection activity and good biocompatibility. Here, we present an overview of the clinically used and tested nanoparticles for imaging and treatment of cancer. Both clinical and preclinical studies show a

variety of the type of nanoparticles developed for cancer. There are more types of nanoparticles currently at an early design step that may progress in the future to preclinical development for cancer imaging and therapy. Nanoparticles for cancer imaging and therapy have evolved rapidly during the last decade and it is expected that more and more will become clinical practise. Their controlled size and multi-functionality are the main reasons for their increasing applications as anticancer agents.

With the continuous development of biotechnology and interdisciplinary penetration and cross each other, also gradually in-depth research on nanometer gene carrier, in the near future, as a new nanometer gene carrier gene delivery carrier will be in medical research and treatment play a bigger role. From all these nanoparticles, we would like to highlight the potential value of theragnostic nanoparticles in both imaging (diagnosis) and therapy (i.e.chemotherapy or genetic therapy).

REFERENCES

- 1 Lindgren M, Rosenthal-Aizman K, Saar K, Eiríksdóttir E, Jiang Y, Sassian M, Ostlund P, Hällbrink M, Langel U. Overcoming metho-trexate resistance in breast cancer tumour cells by the use of a new cell-penetrating peptide. *Biochem Pharmacol* 2006; **71(4)**: 416-425
- 2 Yoshihisa Namiki, Tamami Namiki, Hiroshi Yoshida, Yukiko Ishii, Akihito Tsubota, Shigeo Koido, Kouichi Nariai, Makoto Mitsunaga, Satoru Yanagisawa, Hideyuki Kashiwagi, Yasuo Mabashi, Yoko Yumoto, Sadayori Hoshina, Kiyotaka Fujise, Norio Tada. A novel magnetic crystal-lipid nanostructure for magnetically guided *in vivo* gene delivery. *Nature Nanotechnology* 2009; **4(9)**: 598-606
- 3 Cavazzana Calvom, Thrashier A, Mavilo F, et al. The future of gene therapy. *Nature* 2004; **427(6977)**: 779-781
- 4 Yang Y, Xu Z, Jiang J, Gao Y, Gu W, Chen L, Tang X, Li Y. Poly (imidazole/DMAEA) phosphazene/DNA self-assembled nanoparticles for gene delivery: Synthesis and in vitro transfection. *J Control Release* 2008; **127(3)**: 273-279
- 5 Zhang S, Zhao B, Jiang H, Wang B, Ma B. Cationic lipids and poly-mersmediated vectors for delivery of siRNA. *J Control Release* 2007; **123(1)**: 1-10.
- 6 Ma B, Zhang S, Jiang H, Zhao B, Lv H. Lipoplexmorphologies and their influences on transfection efficiency in gene delivery. *J Control Release* 2007; **123(3)**: 184-194
- 7 Sakaguchi N, Kojima C, Harada A, Koiwai K, Shimizu K, Emi N, Kono K. Enhancement of transfection activity of lipoplexes by complexation with transfer-rin-bearing fusogenic polymer-modified liposomes. *Int J Pharm* 2006; **325(1-2)**: 186-190
- 8 Zhang Y, Li H, Sun J, Gao J, Liu W, Li B, Guo Y, Chen J. DC-Chol/DOPE cationic lipo-somes: A comparative study of the influence factors on plasmid pDNA and siRNA gene delivery. *Int J Pharm* 2010; **390(2)**: 198-207
- 9 Chowdhury EH, Kunou M, Nagaoka M, Kundu AK, Hoshiba T, Akaike T. High-efficiency gene delivery for expression in mammalian cells by nanoprecipitates of Ca-Mg phosphate. *Gene* 2004; **341**: 77-82
- 10 Roy I, Ohulchanskyy TY, Bharali DJ, et al. Optical tracking of or-ganically modified silica nanoparticles as DNA carriers: a nonviral, nanomedicine approach for gene delivery. *Proc Natl Acad Sci USA* 2005; **102(2)**: 279-284
- 11 Qin F, Zhou Y, Shi J, et al. A DNA transporter based on mesoporous sili-ca nanospheres mediated with polycation poly (allylaminehy-drochloride) coating on mesopore surface. *Biomed Mater Res* 2009; **90A**: 333
- 12 Mahmoudi M, Simchi A, Imani M, Häfeli UO. Superparamagnetic iron oxide nanoparticles with rigid cross-linked

- polyethylene glycol fumarate coating for application in imaging and drug delivery. *J Phys Chem* 2009; **113**(19): 8124-8131
- 13 Qureshi HY, Ahmad R, Zafarullah M. High-efficiency transfection of nucleic acids by the modified calcium phosphate precipitation method in chondrocytes. *Anal Biochem* 2008; **382**(2): 138-140
 - 14 Sandhu KK, McIntosh CM, Simard JM, Smith SW, Rotello VM. Gold nanoparticles mediated transfection of mammalian cells. *Bioconjugate Chem* 2002; **13**(1): 3-6
 - 15 Saccardo P, Villaverde A, González-Montalbán N. Peptide-mediated DNA condensation for non-viral gene therapy. *Biotechnology Advances* 2009; **27**(4): 432-438
 - 16 Jackson DA, Juranek S, Lipps HJ. Designing nonviral vectors for efficient gene transfer and long-term gene expression. *Mol Ther* 2006; **14**(5): 613-626
 - 17 Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. *Nat Rev Drug Discov* 2005; **4**(7): 581-593
 - 18 Steele TWJ, Shier WT. Dendrimeric alkylated polyethylenimine nano-carriers with acid-cleavable outer cationic shells mediate improved transfection efficiency without increasing toxicity. *Pharm Res* 2010; **27**(4): 683-698
 - 19 Santos JL, Oliveira H, Pandita D, Rodrigues J, Pêgo AP, Granja PL, Tomás H. Functionalization of poly (amidoamine) dendrimers with hydrophobic chains for improved gene delivery in mesenchymal stem cells. *J Control Release* 2010; **144**(1): 55-64
 - 20 Gu Xiaohu, Xu Liqiang, Tian Fang, Ding Yi. Au-Ag Alloy Nanoporous Nanotubes. *NANO RESEARCH* 2009; **2**(5): 386-393
 - 21 Gu Xiaohu, Cong Xiao, Ding Yi. Platinum-Decorated Au Porous Nanotubes as Highly Efficient Catalysts for Formic Acid Electro-Oxidation. *CHEMPHYSICHEM* 2010; **11**(4): 841-846
 - 22 Cheng Yingwen, Qiu Cuicui, Ma Houyi, Zhang Xiaoka, Gu Xiaohu. Unusual corrosion process of gold nanoplates and the mechanism study. *NANOSCALE* 2010; **2**(5): 685-688
 - 23 Ge Xingbo, Wang Rongyue, Liu Pengpeng, Ding Yi. Platinum-decorated nanoporous gold leaf for methanol electrooxidation. *CHEMISTRY OF MATERIALS* 2007; **19** (24): 5827-5829.
 - 24 Kaul G, Amiji M. Tumor-targeted gene delivery using poly(ethylene glycol)-modified gelatin nanoparticles: in vitro and in vivo studies. *Pharm Res* 2005; **22**(6): 951-961
 - 25 Balogh L, Nigavekar SS, Nair BM. Significant effect of size on the in vivo biodistribution of gold composite nanodevices in mouse tumor models. *Nanomedicine* 2007; **3**(4): 281-296
 - 26 Yang SY, Sun JS, Liu CH. Ex vivo magnetofection with magnetic nanoparticles: a novel platform for nonviral tissue engineering. *Artif Organs* 2008; **32**(3): 195-204
 - 27 Dobson J. Magnetic micro-and nano-particle-based targeting for drug and gene delivery. *Gene Ther* 2006; **1**(1): 31-37
 - 28 Wang ZY, Wang L, Zhang J, Li YT, Zhang DS. A study on the preparation and characterization of plasmid DNA and drug-containing magnetic nanoliposomes for the treatment of tumors. *Inter J Nanomed* 2011; **6**: 871-875
 - 29 Cai JT, Liu HN, Zhang Y, et al. Preparation and characterization of magnetic nanoparticles containing Fe₃O₄-dextran-anti- β -human chorionic gonadotropin, a new generation chori-ocarcinoma-specific gene vector. *Inter J Nanomed* 2011; **6**: 285-294
 - 30 Bhattarai SR, Kim SY, Jang KY. N-hexanoyl chitosan-stabilized magnetic nanoparticles: enhancement of adenoviral-mediated gene expression both in vitro and in vivo. *Nanomedicine* 2008; **4**(2): 146-154
 - 31 Chaekyu Kim, Sarit Agasti, Zhengjiang Zhu, Lyle Isaacs, Vincent M. Rotello. Recognition-mediated activation of therapeutic gold nanoparticles inside living cells. *Nature Chemistry* 2010; **2**(11): 962-966.
 - 32 Nathaniel L. Rosi, David A. Giljohann, C. Shad Thaxton, Abigail K. R. Lytton-Jean, Min Su Han, Chad A. Mirkin. Oligonucleotide-Modified Gold Nanoparticles for Intracellular Gene Regulation. *Science* 2006; **312**(5): 1027-1030
 - 33 Jaswinder Sharma, Rahul Chhabra, Anchi Cheng, Jonathan Brownell, Yan Liu, Hao Yan. Control of Self-Assembly of DNA Tubules Through Integration of Gold Nanoparticles. *Science* 2009; **323**(1): 112-116
 - 34 Byoungjin Kim, Gang Han, Bhushan J. Toley, Chae-kyu Kim, Vincent M. Rotello and Neil S. Forbes. Tuning payload delivery in tumour cylinders using gold nanoparticles. *Nature Nanotechnology* 2010; **5**(6): 465-472
 - 35 Gang Peng, Ulrike Tisch, Orna Adams, Meggie Hakim, Nisreen Shehada, Yoav Y. Broza, Salem Billan, Roxolyana Abdah-Bortnyak, Abraham Kuten and Hossam Haick. Diagnosing lung cancer in exhaled breath using gold nanoparticles. *Nature Nanotechnology* 2009; **4**(10): 669-673
 - 36 Noh S, Kim W, Kim S, Kim J, Baek K, Oh Y. Enhanced cellular delivery and transfection efficiency of plasmid DNA using positively charged biocompatible colloidal gold nanoparticles. *BBA-General Subjects* 2007; **5**: 747-752
 - 37 Note C, Kosmella S, Koetz J. Poly(ethyleneimine) as reducing and stabilizing agent for the formation of gold nanoparticles in w/o microemulsions. *Colloids and Surfaces A: physico-chemical and Engineering Aspects* 2006; (1-3): 150-156
- Peer reviewer:** Gregor Sersa, Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia.