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·综述·

Advance in polyamidoamine dendrimers as gene delivery agents

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Gene therapy recently has become an important area of research as a new therapeutic method. In vivo and in vitro gene therapies require efficient delivery of genetic material into a cell and preferably high levels of expression of transferred gene. Traditionally, gene delivery systems are classified as viral vector-mediated systems and nonviral vector-mediated systems. Viral vectors, which have been demonstrated as systems with high transfection efficiency, however, are limited due to adverse effects such as immunogenicity, toxicity, limited DNA carrying capacity and mutagenesis caused by cell-infected viruses[1]. For these reasons, nonviral systems, especially synthetic DNA delivery systems such as cationic liposomes or synthetic polymers, have become increasingly desirable in both basic research and clinical areas. Liposomes have several potential advantages, including the ability to carry a significant amount of drug, relative ease of preparation and low toxicity if natural lipids are used. However, common problems encountered with liposomes are low stability, poor tissue specificity, toxicity with non-native lipids, and uptake by phagocytic cells, thereby reducing circulation times. Polyamidoamine (PAMAM) dendrimers, a family of branched synthetic polymers, are uniform in size with a high density of amino groups restricted to the surface as well as highly soluble and stable in aqueous solution[2,3]. Experiments showed that PAMAM dendrimers are effective transfection agents, providing high successful rate of transferring genetic materials into the cell. The lack of toxicity, immunogenicity[4,5], stability of DNA/dendrimer complexes and high transfection efficiency suggest that this transfection method may be useful for gene therapy.

I FORMATION OF DENDRIMER-DNA COMPLEX

DNA and PAMAM dendrimers form complexes on the basis of the electrostatic interactions between positively charged amino groups of the dendrimers and negatively charged phosphate groups of the nucleic acid[6,7]. Electron microscopic examination of the complexes indicated that the majority of the plasmid DNA is contracted into isolated toroids. The binding of plasmid DNA to dendrimer appears to alter the secondary and tertiary structure, but does not alter its primary structure. Complexed DNA is protected against degradation by either specific nucleases or cellular extracts containing nuclease activity. The most efficient transfection occurs when the complex is formed in positive charge excess, the dendrimer-DNA charge ratio may be from about 10,000:1 to 10:1 and the proportions can be optimized for a particular application.

II CELL TRANSFECTION WITH DENDRIMERS

DNA-dendrimers complex can transfect an unusually wide range of adherent and nonadherent cells. This attribute is relatively rare among synthetic transfection reagents. PAMAM dendrimers are also superior for transfecting suspension cell lines[8], and compared with many commercially available cationic liposomes, dendrimers have a broader concentration range between transfection and cytotoxicity[9]. Dendrimer mediated transfection of the luciferase gene into Jurkat and U937, both nonadherent cells of limphoid lineage, is one to two orders of magnitute higher than that obtained with the commercial lipids, Lipofect AMINE and DMRIE-C. Another potential advantage of dendrimers in gene delivery lies in their capability of transfecting wide range of primary cells.

III INTRACELLULAR DELIVERY

Cationic dendrimer/DNA complexes may initiate cell entry through binding to anionic phospholipids on the cell membrane and triggering either spontaneous endocytosis or direct plasma membrane disruption. Tracing cell entry mechanisms with DNA and/or dendrimer in the complex indicates that majority of the complexes in most cells are internalized through an energy-dependent endocytosis[10]. The DNA release from the complex may therefore be the result of an exchange with anionic cytoplasmic RNA, proteoglycans, natural polyamines or nuclear chromatin. Since mitotically active cell lines show higher levels of expression of introduced genes, the majority of translocation of exogenous DNA to the nucleus

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may occur during cell division.

IV IN VITRO GENE TRANSFER

In vitro gene transfer using PAMAM dendrimers has recently experienced rapid growth. Kukowska et al[9] examined the efficiency of plasmid DNA-dendrimer transfection using two reporter gene systems: firefly luciferase and bacterial beta-galactosidase. Highly efficient transfection of a broad range of eukaryotic cells and cell lines was achieved with minimal cytotoxicity. The capability of a dendrimer to transfect cells appeared to depend on the size, shape, and generation (i.e. number of primary amino groups on the surface) of the polymer.

In contrast to the other DNA carriers, dendrimer/DNA complexes retain the ability to transfect after drying, which enabled coating or incorporation of complexes into poly(DL-lactide-co-glycolide) or collagen-based biorodable membranes, which provide support for the use of this technology for in vitro and in vivo transfection of skin cells[11]. In contrast to lipofection, gene transfer with PAMAM dendrimers to airway epithelial cells is not inhibited by pulmonary surfactant in vitro. PAMAM dendrimers appear to be the more stable gene delivery systems for treatment of lung diseases by into the airways[12].

Hudde et al[13] investigated the efficiency of activated polyamidoamine dendrimers to transfect rabbit and human corneas in vitro culture. The results showed that activated dendrimers are an efficient nonviral vector capable of transducing corneal endothelial cells in vitro, which may have applications in gene-based approaches aimed at prevention of corneal allograft rejection or in treatment of other disorders of corneal endothelium.

A surprising discovery is that the transfection activity of the dendrimers is dramatically enhanced (> 50-fold) by heat treatment[14]. Such treatment induces significant degradation of the dendrimer at the amide linkage, resulting in a heterodisperse population of compounds with molecular weights ranging from the very low (< 1500 Da) to several tens of kilodaltons. Transfection activity is related both to the initial size of the dendrimer and its degree of degradation. The increased transfection after the heating process is principally due to the increase in flexibility that enables the fractured dendrimer to be compact when complexed with DNA and swell when released from DNA. Turunen et al[15] tested different cationic polymers in vitro for their ability to transfect rabbit aortic smooth muscle cells and human endothelial cells with lacZ marker gene. Fractured PAMAM dendrimers had the highest in vivo gene transfer efficiency and no signs of inflammation were seen, so fractured PAMAM dendrimer can be used for arterial gene therapy via adventitial gene delivery route. Urtti et al[16] evaluated the efficacy of several synthetic DNA complexing compounds in transfecting primary human retinal pigment epithelial (RPE) cells in vitro. Degraded PAMAM dendrimers exhibited the best combination of high activity and low toxicity in RPE cell transfection.

PAMAMs as well may be as a potential delivery vehicle for oligonucleotides[17]. Dendrimer-oligonucleotide complexes were stable in 50 % serum to variations in pH and ionic strength. Using dendrimers resulted in a 50-fold enhancement in cell uptake of oligonucleotide as determined by flow cytometry, and enhanced cytosolic and nuclear availability as shown by confocal microscopy. These data support the further evaluation of dendrimers for oligonucleotide delivery in cell culture and in vivo.

V IN VIVO GENE TRANSFER

Trials are currently being carried out to assess the efficacy of in vivo gene delivery using dendrimers.

Maruyama's[18] study demonstrates in vivo effectiveness of Epstein-Barr virus (EBV)-based plasmid vector coupled with PAMAM dendrimer in suicide gene therapy of cancer. HSV-1 tk gene was transferred into Ewing's sarcoma cell lines, A4573 and KP-EWS-YI, by using an EBV-based plasmid vector. The treatment with pSES. Tk/dendrimer also resulted in significant prolongation of survival of the mice implanted with A4573. They also applied this method to express suicide gene in hepatocellular carcinoma cells[19].

PAMAM dendrimer was investigated to augment plasmid-mediated gene transfer efficiency in a murine cardiac transplantation model[20]. The use of the PAMAM dendrimer dramatically increased the efficiency of plasmid-mediated gene transfer and expression. Production of immunosuppressive cytokines at higher amounts for longer periods of time in a greater expanse of tissue enhanced the immunosuppressive effect and prolonged graft survival further.

VI DELIVERY OF ANTISENSE REGULATORY NUCLEIC ACIDS

Antisense oligonucleotides can be trandferred into cells to block the production of specific proteins. This may be useful in suppressing cells that grow abnormally, such as cancer cell, or in the alteration of normal cell functions, such as immunosuppression for organ transplantation. PAMAM dendrimers function as an effective delivery system for the introduction of regulatory nucleic acids and facilitate the suppression of the specific gene expression.

Yoo et al[21] used HeLa cells transfected with plasmid pLuc/705 which has a luciferase gene interrupted by a human beta-globin intron mutated at nucleotide 705, thus causing incorrect splicing. As a result, PAMAM dendrimers formed stable complexes with oligonu-

cleotides that had modest cytotoxicity and showed substantial delivery activity. The dose of the oligonucleotide, the charge ratio of oligonucleotide to dendrimer, and the size of the dendrimers were all critical variables for the antisense effect. Compared to other types of delivery agents, PAMAM dendrimers were more effective in delivering oligonucleotides into the nucleus of cells in the presence of serum proteins. In addition, Yoo et al[22] prepared dendrimers conjugated with the fluorescent dye Oregon green 488. The 2'-O-methyl antisense oligonucleotide sequence used in these studies was designed to correct splicing at an aberrant intron inserted into a luciferase reporter gene. A surprising result of these studies was that the Oregon green 488-conjugated dendrimer was a much better delivery agent for antisense compounds than unmodified dendrimer. This suggests that coupling of relatively hydrophobic small molecules to PAMAM dendrimers may provide a useful means of enhancing their capabilities as delivery agents for nucleic acids.

Bielinska et al[23] investigated the ability of PAMAM dendrimers to function as an effective delivery system for antisense oligonucleotides and 'antisense expression plasmids' for the targeted modulation of gene expression. They developed the cell lines that permanently express luciferase gene. Transfections of antisense oligonucleotides or antisense cDNA plasmids into these cell lines using dendrimers resulted in a specific and dose dependent inhibition of luciferase expression. This inhibition caused approximately 25%-50% reduction of baseline luciferase activity. Binding of the phosphodiester oligonucleotides to dendrimers also extended their intracellular survival.

Helin et al[24] used PAMAM dendrimer to bind oligodeoxynucleotides (ODNs) electrostatically, the complexed phosphodiester ODNs were protected from nuclease degradation and also increased their cellular uptake and pharmacological effectiveness, antisense sequence-specific inhibition of more than 70% were obtained.

VII SUMMARY AND PROSPECT

The unique structure of PAMAM dendrimers validates their proposed role as gene delivery agents. In this review, we outline the transfection mechanisms and the ability of Starburst dendrimers to mediate high efficiency transfer of genetic material in a wide variety of cell lines in vitro and in vivo. Moreover, PAMAM dendrimers could also be applied in other gene transfection techniques, such as particle bombardment and as enhancers of retroviral transduction[25]. Since antibody-conjugated and folate-conjugated dendrimers have already been used experimentally in immunoassays[26] in the imaging of tumors[27] respectively, it is possible that similar forms may be useful in targeted gene delivery in vivo, which could enable various therapeutic uses of gene transfer. PAMAM dendrimers as gene delivery agents have experienced rapid development, there is no doubt they will continue to yield wonderful fruits.

References

[1] Crystal RG. Transfer of genes to humans: early lessons and obstacles to success[J]. Science, 1995, 270: 404—410.

[2] Tomalia DA, Baker H, Dewald J, et al. A new class of polymers: starburst-dendritic macromolecules[J]. J Polymer, 1985, 17(1): 117—132.

[3] Tomalia DA, Naylor AM, Goddard WA. Starburst dendrimer: molecular-level control of size, shape, surface chemistry, topology, and flexibility from atoms to macroscopic matter[J]. Angew Chem Int, Ed Engl, 1990, 29: 138—175.

[4] Malik N, Wiwattanapatapee R, Klopsch R, et al. Dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo[J]. J Controlled Release, 2000, 65: 133—48.

[5] Roberts JC, Bhalgat MK, Zera RT. Preliminary biological evaluation of polyamidoamine (PAMAM) Starburst dendrimers [J]. J Biomed Mater Res, 1996, 30(1): 53—65.

[6] Bielinska AU, Kukowska JF, Baker JR Jr. The interaction of plasmid DNA with polyamidoamine dendrimers: mechanism of complex formation and analysis of alterations induced in nuclease sensitivity and transcriptional activity of the complexed DNA[J]. Biochem Biophys Acta, 1997, 1353(2): 180—90.

[7] Bielinska AU, Chen C, Johnson J, et al. DNA complexing with polyamidoamine dendrimers: implications for transfection [J]. Bioconjug Chem, 1999, 10(5): 843—50.

[8] Haensler J, Szoka FC Jr. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture[J]. Bioconjug Chem, 1993, 4(5): 372—9.

[9] Kukowska Latallo JF, Bielinska AU, Johnson J, et al. Efficient transfer of genetic material into mammalian cells using Starburst polyamidoamine dendrimers[J]. Proc Natl Acad Sci USA, 1996, 93(10): 4897—902.

[10] Zanta MA, Boussif O, Adib A, et al. In vitro gene delivery to hepatocytes with galactosylated polyethtlenimine[J]. Bioconjug Chem. 1997, 8(6): 839—44.

[11] Bielinska AU, Yen A, Wu HL, et al. Application of membrane-based dendrimer/DNA complexes for solid phase transfection in vitro and in vivo[J]. Biomaterials, 2000, 21(9): 877—87.

[12] Ernst N, Ulrichskotter S, Schmalix WA, et al. Interaction of liposomal and polycationic transfection complexes with pulmonary surfactant[J]. J Gene Med, 1999, 1(5): 331—40.

[13] Hudde T, Rayner SA, Comer RM, et al. Activated polyamidoamine dendrimers, a non-viral vector for gene transfer to the corneal endotheliu m[J]. Gene Ther, 1999, 6(5): 939—43.

[14] Tang MX, Redemann CT, Szoka FC Jr. In vitro gene delivery by degraded polyamidoamine dendrimers[J]. Bioconjug Chem, 1996, 7(6): 703—14.

[15] Turunen MP, Hiltunen MO, Ruponen M, et al. Efficient adventitial gene delivery to rabbit carotid artery with cationic

polymer-plasmid complexes[J]. Gene Ther, 1999, 6(1): 6—11.

[16] Polansky J, Lui GM, Szoka FC. Gene delivery and expression in human retinal pigment epithelial cells: effects of synthetic carriers, serum, extracellular matrix and viral promoters [J]. J Drug Target, 2000, 7(6): 413—21.

[17] Delong R, Stephenson K, Loftus T, et al. Characterization of complexes of oligonucleotides with polyamidoamine starburst dendrimers and effects on intracellular delivery[J]. J Pharm Sci, 1997, 86(6): 762—4.

[18] Maruyama Tabata H, Harada Y, Matsumura T, et al. Effective suicide gene therapy in vivo by EBV-based plasmid vector coupled with polyamidoamine dendrimer[J]. Gene Ther, 2000, 7(1): 53—60.

[19] Harada Y, Iwai M, Tanaka S, et al. Highly efficient suicide gene expression in hepatocellular carcinoma cells by Epstein-Barr virus-based plasmid vectors combined with polyamidoamine dendrimer[J]. Cancer Gene Ther, 2000, 7(1): 27—36.

[20] Qin L, Pahud DR, Ding Y, et al. Efficient transfer of genes into murine cardiac grafts by Starburst polyamidoamine dendrimers[J]. Hum Gene Ther, 1998, 9(4): 553—60.

[21] Yoo H, Sazani P, Juliano RL. PAMAM dendrimers as delivery agents for antisense oligonucleotides[J]. Pharm Res, 1999, 16(12): 1799—804.

[22] Yoo, H, Juliano RL. Enhanced delivery of antisense oligonucleotides with fluorophore-conjugated PAMAM dendrimers[J]. Nucleic Acids Res, 2000, 28: 4225—4231.

[23] Bielinska A, Kukowska Latallo JF, Johnson J, et al. Regulation of in vitro gene expression using antisense oligonucleotides or antisense expression plasmids transfected using starburst PAMAM dendrimers[J]. Nucleic Acids Res, 1996, 24(11): 2176—82.

[24] Helin V, Gottikh M, Mishal Z, et al. Cell cycle-dependent distribution and specific inhibitory effect of vectorized antisense oligonucleotides in cell culture[J]. Biochem Pharmacol, 1999, 58(1): 95—107.

[25] Hodgson CP, Xu G, Solaiman F, et al. Progress toward biosynthetic retrovectoring systems for gene therapy[J]. J Mol Med, 1997, 75:249—258.

[26] Singh P, Moll F 3rd, Lin-SH, et al. Starburst dendrimers: enhanced performance and flexibility for immunoassays[J]. Clin Chem, 1994, 40(9): 1845—9.

[27] Konda SD, Aref M, Brechbiel M, et al. Development of a tumor-targeting MR contrast agent using the high-affinity folate receptor: work in progress[J]. Invest Radiol, 2000, 35(1): 50—7.

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