REVIEWS

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Gene Therapy Prospects – Intranasal Delivery of Therapeutic Genes

Perspektywy terapii genowej – donosowe podawanie terapeutycznych genów

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Abstract

Gene therapy is recognized to be a novel method for the treatment of various disorders. Gene therapy strategies involve gene manipulation on broad biological processes responsible for the spreading of diseases. Cancer, monogenic diseases, vascular and infectious diseases are the main targets of gene therapy. In order to obtain valuable experimental and clinical results, sufficient gene transfer methods are required. Therapeutic genes can be administered into target tissues *via* gene carriers commonly defined as vectors. The retroviral, adenoviral and adeno-associated virus based vectors are most frequently used in the clinic. So far, gene preparations may be administered directly into target organs or by intravenous, intramuscular, intratumor or intranasal injections. It is common knowledge that the number of gene therapy clinical trials has rapidly increased. However, some limitations such as transfection efficiency and stable and long-term gene expression are still not resolved. Consequently, great effort is focused on the evaluation of new strategies of gene delivery. There are many expectations associated with intranasal delivery of gene preparations for the treatment of diseases. Intranasal delivery of therapeutic genes is regarded as one of the most promising forms of pulmonary gene therapy research. Gene therapy based on inhalation of gene preparations offers an alternative way for the treatment of patients suffering from such lung diseases as cystic fibrosis, alpha-1-antitrypsin defect, or cancer. Experimental and first clinical trials based on plasmid vectors or recombinant viruses have revealed that gene preparations can effectively deliver therapeutic or marker genes to the cells of the respiratory tract. The noninvasive intranasal delivery of gene preparations or conventional drugs seems to be very encouraging, although basic scientific research still has to continue (**Adv Clin Exp Med 2012, 21, 4, 525–534**).

Key words: gene preparations, gene therapy, inhalation, cystic fibrosis, alpha-1-antitrypsin deficiency, lung cancer.

Streszczenie

Terapia genowa jest nową metodą leczenia chorób. Strategia terapii genowej opiera się na wykorzystaniu do celów terapeutycznych genów kodujących białka o kluczowym znaczeniu dla przebiegu procesów biologicznych warunkujących powstawanie i rozwój chorób. Obecnie próby kliniczne terapii genowej dotyczą głównie nowotworów, zaburzeń sercowo-naczyniowych i chorób zakaźnych. Skuteczność kliniczna terapii genowej wiąże się bezpośrednio z opracowywaniem metod wprowadzania genów do wybranych komórek, narządów. Terapeutyczne geny są dostarczane do tkanek za pomocą nośników – wektorów. Retrowirusy, adenowirusy i wirusy związane z adenowirusami są najczęściej stosowanymi wektorami terapii genowej w klinice. Dotychczas preparaty genowe mogą być podawane dożylnie, domięśniowo, dojamowo, doguzowo oraz donosowo. Skuteczność terapeutyczna genów, terapii genowej jest bezpośrednio związana z ekspresją transgenów w docelowych komórkach. Zarówno zakłady badań podstawowych, jak i kliniki nie dysponują jeszcze optymalnymi wektorami, metodami wprowadzania genów do chorych narządów. Wiele grup badawczych prowadzi badania poświęcone udoskonalaniu istniejących i poszukiwaniu nowych metod wprowadzania terapeutycznych genów do komórek. Pomysł wprowadzania terapeutycznych genów przez nos jest bardzo interesujący głównie z uwagi na olbrzymią aplikacyjność kliniczną. Donosowe dostarczanie terapeutycznych genów jest uważane za jedną z najbardziej obiecujących – bezpiecznych i skutecznych metod w terapii genowej chorób płuc. Terapia genowa wykorzystująca preparaty genowe wprowadzane przez nos pozwala podejmować próby leczenia pacjentów cierpiących np. na mukowiscydozę, defekt alfa-1-antytrypsyny lub nowotwory. Badania eksperymentalne i pierwsze próby kliniczne przeprowadzone z wektorami plazmidowymi lub rekombinowanymi wirusami wykazały,

że stosowane wektory mogą wydajnie dostarczać terapeutyczne geny do komórek układu oddechowego. Wykorzystanie donosowej, nieinwazyjnej drogi podawania preparatów genowych lub konwencjonalnych leków budzi entuzjazm wśród naukowców i pacjentów. Dalsze badania określą bezpośrednią użyteczność i dostępność donosowych preparatów genowych w klinice (**Adv Clin Exp Med 2012, 21, 4, 525–534**).

Słowa kluczowe: preparaty genowe, terapia genowa, wdychanie, mukowiscydoza, defekt alfa-1-antytrypsyny, rak płuc.

The increasing number of gene therapy clinical trials over the last two decades is easily observed. However, despite preliminary encouraging results, gene therapy is still recognized as an experimental method not widely accepted for clinical use, mainly because of technical limitations. The currently available clinical gene preparations and gene delivery methods do not guarantee efficient delivery of therapeutic genes to the target tissue and long-term and stable gene expression. However, extensive research projects have been initiated in recent years as a result of a well-described number of new therapeutic genes and gene-delivery vector systems. Inhalation (instillation) techniques have been proposed as promising methods of gene administration to the lungs. It is common knowledge that, for example, aerosol drug delivery is extensively used in the treatment of respiratory diseases and therefore is described as an effective, non-invasive and safe therapeutic approach. Therefore, the idea of gene therapy based on inhalation is conducive to diseases of the airway, especially for patients suffering from cystic fibrosis, alpha-1-antitripsin deficiency, bronchial asthma, lung cancer or infectious diseases. This review presents the actual knowledge concerning inhalation gene therapy strategies with special focus to their clinical applications.

Gene Preparations

Gene preparations containing nucleic acids are delivered to target tissues by carriers classified into two major types, virus and non-virus. It has been convincingly demonstrated that plasmid DNA as well as recombinant viruses could be efficiently introduced into the airways *via* either aerosol inhalation and intranasal instillation *via* syringe or a catheter [1–12]. Intranasal administration enables gene delivery to target cells in the nasal cavity as well as in the lungs. The relatively large surface of nasal epithelium, pronounced vascularity of nasal mucosa with high total blood flow and porous structure of the endothelial membrane enable efficient gene delivery directly into the bloodstream and lungs. One of the main advantages of the inhaled administration of gene preparations over systemic delivery (e.g. intravenous injection) is most certainly its non-invasiveness. It also allows more efficient gene deposition within the respiratory system due to liver (Kupffer cells) extraction avoidance [13]. Liu et al. [13] demonstrated that though intravenous injection of the p53-PEI complex resulted in a threefold higher number of human p53 plasmid copies than aerosol inhalation, the lung retention time for the injected p53 gene was significantly shorter than for its inhaled form. Almost 80% of human p53 was cleared from the mouse lungs within 10 minutes after intravenous deposition. Significant differences in biodistribution were also documented. Inhaled human p53 plasmid was detected mainly in the lungs, whereas intravenous administration resulted in its high concentrations in the liver, spleen and blood [13]. It should be mentioned as well that aerosol administration allows a fairly even distribution of inhaled material throughout the airways. That obviously enhances its clinical effectiveness, as direct deposition of genetic material in a relatively small area of the lung epithelium has been shown to induce both inflammatory response and clearance mechanisms [3]. The main shortcomings of inhalation gene delivery are mostly attributed to the excessive expenditure of the gene preparation (e.g. by exhalation) as well as the difficulties in assessing the exact lung-deposited dose. This implies the administration of concentrated preparations in high doses and therefore considerably increases costs [2].

There is a variety of commercially available devices for the inhalation procedure and intranasal aerosol delivery. The nose-only exposure chambers are usually employed for animal studies [14]. In humans, nasal masks or nasal interfaces are the commonly used devices, while aerosol is usually generated by nebulizers, mostly jet nebulizers, vibrating mesh nebulizers and ultrasonic nebulizers as well as electrostatic spray [15–19]. The device choice should be determined by the physicochemical properties of the gene preparation (e.g. density, droplet size, charge, solubility, etc.) but also by clinical indication, cost and functionality. Moreover, the nebulizer technical characteristics, like the setup conditions, aerosol concentration and rate are of considerable importance. All these directly affect the parameters critical for the effectiveness of aerosol administration

– fluid flow and size of the lung-deposited aerosol fraction as well as the medication's bioavailability. Typically for nebulization, the high degradation rate of gene preparations caused by the hydrodynamic share forces is thought as the main disadvantage [18, 19]. As much as 70–90% of the naked plasmid DNA might be degraded by share forces in a jet nebulizer, vibrating mesh nebulizer or ultrasonic nebulizer, whereas the electrostatic spray was shown to maintain the majority of the initial supercoiled DNA (CCC form) [19]. Consequently, nebulizers are not recommended for the delivery of suspensions containing naked plasmid. Meanwhile, several methods of plasmid DNA protection have been developed, mostly exploiting its ability to complex to cationic polymers and lipids. The most commonly used non-viral carriers are the cationic polymer of polyethylenimine (PEI) and cationic lipid GL-67 (N4-spermine cholesterylcarbamate) characterized by high stability during the atomization process [17, 20].

Droplet size and stability should also be mentioned as the most important factors influencing the efficacy of aerosol inhalation. The bioavailability of particles with a size bigger than 5 μm is low due to poor absorption and mucocilliary clearance in the airways. Meanwhile, particles with a diameter up to 1 μm are exhaled and not deposited in the lungs. Therefore, the optimal aerosol particle is generally within the 1–5μm size range [16, 21, 22]. Along with intransal inhalation, other techniques of gene delivery to the lungs have been investigated. For example, it has been suggested that intratracheal administration might be more effective due to the direct deposition into the lungs [6, 11, 22]. However, Zou et al. [21] observed that intranasally delivered *p53*-specific luciferase plasmid complexed with L-polylysine was expressed significantly, 1.6-fold, higher than after intratracheal administration. Besides, intratracheal administration is an invasive method demanding specialized devices and surgical intervention, costly and with much higher risk of side effects due to the strong immune and inflammatory response [11].

Gene Carriers

There are no ideal vectors allowing efficient gene deposition in the respiratory system. Great effort is being focused on cloning virus or non-virus vectors as therapeutic gene carriers. The recombinant virus vectors or non-virus vectors most frequently are directed at the ciliated epithelial cells, alveolar pneumocytes and cancer cells. Still, an optimal gene delivery method is selected not only in consideration of targeted cell type. The gene preparation characteristic itself, technical abilities of gene therapy laboratories and clinics also have to be considered. Specific conditions present within the respiratory tract due to the underlying disease, such as abnormal cellular surfactant synthesis, mucus layer thickness, or active local inflammation have to be acknowledged as well.

Adenovirus Vectors

Recombinant adenovirus vectors (rAdV) characterized by high transduction efficiency are employed to deliver genes to a variety of cells. The natural tropism to the lung tissue makes rAdV well suited for pulmonary gene therapy. However, the studies on animal models show that intranasal delivery of the replication-defective recombinant adenovirus vector not only resulted in a relatively high gene expression in the lungs, olfactory bulbs and trachea, but also in considerable inflammatory response within the respiratory system and to a lesser extent in the liver [10]. Although, the animal studies indicate that Ad vector administration to the respiratory epithelial surface may generate host anti-vector immune responses seems to be convincing, it is worth mentioning that Harvey et al. [23] published studies describing some differences in the immune response of humans and mice to inhaled rAdV vectors. Authors reveal both the strengths and the limits of using experimental animals to predict human responses to gene vectors. They describe that respiratory epithelial administration of first-generation Ad vectors at doses up to 10^{10} pu to the airway epithelium of healthy individuals elicits minimal to no detectable systemic and mucosal humoral and cellular immune responses. This observation was in opposition to the well-known host responses measured in animals [23].

Adeno-Associated Virus Vectors

Vectors established on an adeno-associated virus (AAV) are of particular value for respiratory applications. There are numerous serotypes capable of inducing high-level, long-lasting transgene expression without any symptoms of specific immune response (e.g. AAV2, AAV5, AAV6, AAV7, AAV8, AAV9) [6, 12, 22, 24]. In animals, recombinant AAV vectors such as rAAV1, rAAV6 and rAAV9 administered by intranasal instillation have been shown to transduce effectively to the nasal airway epithelium. Interestingly, rAAV1-mediated transgene expression was detected mainly

within mouse bronchial epithelium, rAAV6 within both bronchial and alveolar epithelium, while rAAV9 was transduced mainly in the alveolar epithelium [12]. The rAAV9 vector was also detected in other tissues like the liver, lymph nodes and spleen, though in much limited quantities [6]. The stable rAAV-mediated transgene expression in the lungs has been shown to persist up to 12 months, therefore proving the involvement of a progenitor airway cell population [6]. Although, the rAAV9 might be re-administered as early as 1 month after previous exposure followed by insignificant transgene expression, repeated administration is usually limited by the neutralizing antibody response to the viral components of the vector [6].

Lentivirus Vectors

Recombinant lentivirus vectors (rLtV) offer many advantages as a gene transfer tool. The unique ability to integrate into the genome of non-dividing cells enables their relatively long and stable transgene expression. In contrast, other retroviruses only transduce dividing cells and, for technical reasons, the high titers preferred for inhalation are difficult to achieve [25]. Finally, genes encoding proteins responsible for specific-immune host response are deleted in lentivirus vectors, therefore their immunogenicity is significantly reduced.

rLtV vectors such as HIV or/and FIV pseudotyped with envelope glycoproteins derived from the filoviruses (Ebola, Marburg), baculoviruses, rhabdoviruses, influenza viruses or coronaviruses have recently been adapted as gene delivery carriers mainly on account of their ability to transduce airway epithelium *via* the apical surface [5, 8, 11, 26]. Most interestingly, the pseudotyped Ebola and Marburg lentiviruses were reported to efficiently transduce mouse and human airway epithelia without disruption of intercellular tight junction integrity [26]. Also, lentiviruses pseudotyped with the baculovirus GP64 envelope fusion protein allow for efficient and persistent transgene expression for about 1 year both in the nasal (respiratory and olfactory) as well as in the bronchial epithelial cells [11, 26]. Repeated intranasal administration of the GP64-FIV vector without significant decline in transgene expression has been also proven feasible. Sinn et al [11] obtained stable and long-lasting (about 1.5 years) gene expression in mice *via* seven intranasal doses of the GP64-FIV-*Luc* vector whereas intravenous re-administration of the GP64-FIV vector was impossible due to the strong specific immune response following its first dose.

Non-Virus Vectors

Intranasal administration of naked plasmid DNA (pDNA; an example of a non-virus vector), pure or complexed with a chemical cationic carrier, leads to the efficient introduction of genes into the lung cells. Similarly to virus vectors, pDNA transfection of targeted cells depends on the gene preparation characteristics and means of administration. Accordingly, intranasal instillation of naked plasmid DNA (or complexed with linear polyethylenoimine 22kDa) instigates transfection in bronchial (ciliated cells and Clara cells) and to a lesser extent alveolar epithelial cells (mainly pneumocytes type II). Similarly, administration of pDNA complexed with poliethylenoimine or liposomes GL67 resulted in visible transfection of alveolar epithelium, mainly type I pneumocytes and, to a lesser extent, type II. In bronchial ciliated and Clara cells, only minor expression was observed [3, 27]. By contrast, intranasal installation of pDNA complexed with lipofectine and copolimeric micelles produced transfection of mainly pneumocytes type II [28].

Physical Methods

The efficiency of gene delivery into a target cell might be improved by physical methods. It has been shown that intranasal instillation of a gene followed by electroporation increased transgene expression in mouse lungs, mostly pneumocytes type I and II, up to 400 fold. However, the target gene expression was unstable [9]. Interestingly, intranasal administration of naked plasmid DNA combined with electroporation enabled higher gene expression as compared to electrotransfection of pDNA complexed with lipids (GL67) and polymers (PEI) (respectively 12- and 360-fold lower). Thus, ultrasonic treatment allowed a 15-fold increase of transgene lung expression in mice with no extra gain when compared to pDNA complexed with GL67 and/or PEI [9]. The implementation of a magnetic field did not augment the final lung expression of plasmid DNA complexed with lipofectamine or GL67, although *in vitro* studies had suggested a substantial increase in gene transfer efficiency after magnetofection. Therefore, it seems very likely that while physical methods increase *in vivo* transfection of naked plasmid DNA, the transfection of DNA complexed with cationic chemical carriers is not affected at all. Finally, it should be mentioned that the relevance of physical methods is diminished by their relative invasiveness resulting for example in local inflammatory response induced by gene transfer.

Barriers to Intranasal Gene Delivery

Multiple physical and biological barriers limit the effectiveness of gene lung delivery. The mucus layer within bronchi slows down absorption and facilitates particle degradation by enzymes and the mucociliary clearance. Also, the polarized structure of airway epithelium significantly affects effective gene delivery. The tightness of intracellular junctions constrains access of inhaled particles to the basolateral membrane – primary location of many receptors involved in virus transmembrane crossing (e.g. for rAdV, rAAV) [29]. Fortunately, pretreatment with mucolytics has demonstrated its effectiveness in improving epithelial gene transfer *via* the transient disruption of the tight junction integrity [12]. Likewise, methylcellulose prevents fast mucociliary clearance and proteasome inhibitors increase apical surface permeability [26]. Interestingly, pretreatment with doxorubicin was proven to increase delivery outcome of both non-virus and virus gene carriers [13, 24]. Ultimately, the newly developed rLtV vectors and rAAV serotypes (e.g. AAV8, AAV9) approach the airway epithelium from the apical site and therefore are not affected by the abovementioned limitations [5, 6, 8, 11, 22].

The transduction outcome might also be disturbed by the specific immune response directed towards virus components, most particularly instigated by repetitive applications of gene carriers. It has been proven that successive doses of rAdV and rAAV vectors might induce a strong immune reaction prompting significant decline in transgene expression [30]. Up to about 95% reduction in gene expression was observed after repeated AdV-*LacZ* administration in C57BL/6 mice alongside a rise of the neutralizing antibodies and pro-inflammatory cytokines (IL-4, IL-5, IL-11, TNFα) levels [31].

Certain innovative techniques developed recently make it possible to mask viral epitopes and therefore avoid or decrease host immune response. Thus, polymer implementation facilitated effective transduction of the airways with similarly successful re-administration with no clinically significant transgene-specific T-cell activation [31]. Likewise, some chemical modifications, such as incorporation of functional chemical groups or alternatively cross-linking with co-polymers, make it possible to decrease the toxicity of cationic polymer/DNA complexes [4, 14, 28, 32]. The new gene carrier formula, an aerosol containing nano-sized glucosylated polyethyleneimine (gPEI), proved its high transfection efficiency and low toxicity in mice allowing successful gene delivery to the lung by intranasal administration [14]. Chitosan/DNA complexes modified with urocanic acid or thioglycolic acid also exhibited a significantly better gene transfer due to their low toxicity and ability to bind and protect plasmid DNA from degradation by nucleases [4, 32]. Finally, an aerosol containing pDNA encoding *p53* complexed with polylysine/ /protamine proved much more effective in transfection of the non-small cell lung carcinoma cells in mice than PEI or cationic lipid formulations (3- to 17-fold, respectively) [21].

Davies et al. [3] have recently proposed that the excess of cationic polymers or lipids might affect the toxicity of gene preparations. Thus, aerosols containing pDNA/PEI complexes allow about 15-fold higher transgene expression in mice and sheep providing that, prior to that, they have been purified and concentrated by ultrafiltration [3].

Quite predictably, effective and sustained gene expression differs along with the pDNA structure [33]. Vectors including specific bacterial sequences like unmethylated CG dinucleotides (CpG) have been shown to induce specific immune and inflammatory response. Consequently, their transfection rate is significantly lower than for the CpG-free pDNA vectors [34].

Finally, appropriate promoter selection allows for improved gene expression in the respiratory system. There are certain lung-specific endogenous promoters like the human polyubiquitin C (UbC) promoter [33]. However, comparable affinity might be achieved *via* appropriately designed genetic modifications, like the introduction of certain mutations into the promoter sequence or high affinity ligands selectively binding to particular receptors [22]. Eventually, the addition of enhancer sequences as signal DNA or RNA sequences as well as signal sequences facilitating nuclear transport have proven effective [35].

Inhalation Gene Preparations – Therapeutic Approaches

Aerosolized gene preparations offer new, challenging options for the clinical application of gene therapy in respiratory diseases, mainly monogenic like cystic fibrosis or alpha-1- antitrypsin deficiency, but also other much more common examples like bronchial asthma or lung cancer [4, 14, 20, 28, 36, 37, 39–42]. Additionally, effective implementation of inhalation gene preparations for gene therapy of non-respiratory diseases has also been demonstrated. Respiratory mucosa enables successful transduction and gene expression followed by efficient

therapeutic protein secretion. In addition, its considerable area abundantly perfused by peripheral blood makes protein absorption easy and efficient. Accordingly, it has been proven in a mouse model that repeated intranasal administration of an rLtV vector containing plasmid DNA with sequence encoding erythropoietin resulted in a significant increase of hematocrit in peripheral blood [11].

Cystic Fibrosis

Cystic fibrosis is one of the most common monogenic diseases with mutations affecting gene encoding of the membrane chloride channel CFTR (cystic fibrosis transmembrane conductance regulator) that determines transmembrane chloride transport in the cells. Consequently, both preclinical and clinical gene therapy studies have aimed at effective transduction of the *CFTR* gene correct copy. The first clinical trial on humans was performed in 1993 with an rAdV vector bearing a *CFTR* gene administered to three patients with successful normalization of chloride ion transport in transduced epithelial cells and no apparent side effects [36]. Up to now, 25 clinical trials with 450 participants have been performed, including 17 studies with intranasal administration of gene preparation. Both, virus (rAdV, rAAV) and non-virus (lipofection, naked pDNA) vectors were used, achieving at least partial re-establishment of membrane chloride channel function. However, in any of those studies, gene therapy triggered clinically significant changes in sodium cell absorption [20]. Thus, the efficiency of therapeutic gene transduction into target epithelial cells was unsatisfactory. Gene transfer methods (due to induction of specific immunity response) and additional barriers specific to cystic fibrosis (*inter alia* excess of dense mucus and strong inflammatory condition) have been mentioned as the main difficulties impeding effective penetration. Consequently, gene carriers more efficient than rAdV and rAAV vectors have been proposed i.e. rLtV vectors or rSendai virus (rSeV) vectors [37]. rSeV allows particularly high efficiency of gene transfer due to its affinity to sialic acid receptors localized on the apical site of respiratory epithelium. Thus, rSeV-CFTR prompts the activation of a biologically functional glycosylated CFTR channel localized at the apical membrane of epithelial cells. This characteristic identifies the rSeV vector as primarily predisposed to gene therapy of cystic fibrosis [38]. In animal experiments, intranasal rSeV administration achieved efficient transduction of nasal epithelial cells, high transgene expression and adequate activity of the CFTR chloride ion channel. Most importantly, significant decrease in sodium absorption has been observed. However, relatively high toxicity and local inflammatory response have been noted as the main side effects [43].

Alpha-1-Antitrypsin Deficiency

Another relatively common monogenic disease affecting the respiratory tract is the hereditary alpha-1-antitrypsin deficiency caused by mutation in serpina-1 gene encoding for the serine protease inhibitor (AAT). Experimental studies in mice have proven that intranasal administration of rAAV vectors encoding for AAT allowed effective transgene expression in the lungs and consequently a significant increase of alpha-1-antitrypsin concentration in broncho-alveolar lavage fluid [6, 22]. Similarly optimistic results have been achieved in human clinical studies with intranasally deposited pDNA encoding for an *AAT* gene complexed with cationic lipids. Importantly, most markers of chronic airway inflammation, typically observed in *AAT*-deficient patients, were considerably down-regulated in the intranasally treated group in comparison to those receiving intravenous AAT protein [44]. However, the relative normalization of AAT protein production as a result of gene therapy did not in any way affect the activity of the mutated Serpina-1 gene and synthesis of the defective endogenous AAT enzyme. Therefore, except for the low serum AAT concentration, other pathological processes were not influenced by the therapeutic *AAT* gene transfection. For that reason, several groups attempted to shut down the mutated gene either by means of silencing and/or mutation correction [45, 46]. While the latter produced somewhat disappointing results mostly due to the technical obstacles, the inhibition of gene expression by siRNA came out as a promising method potentially applicable not only in alpha-1-antitrypsin deficiency, but also other persistent inflammatory diseases, for example COPD. Significant, up to 40%, down-regulation of defective gene expression was observed in bronchial epithelial cells after intranasal administration of siRNA complexed with chitosan [47]. Moreover, it was also demonstrated that siRNA might be installed in target lung cells both by virus vectors or chemical carriers [48].

Lung Cancer

Apart from the treatment of inherited genetic diseases, the effectiveness of intranasally delivered gene preparations has also been extensively

evaluated in lung cancer, mostly as a tool aiming at tumor cell elimination, tumor growth inhibition and metastasis formation. In comparison to other methods of gene deposition, like intravenous, intratumoral, intrabronchial or intrapleural, the application into the nasal cavity is obviously more convenient and at least equally effective. It has been clearly demonstrated that intranasal gene delivery allowed efficient transfection of lung cells adequate enough to achieve therapeutic effect. Moreover, nasal delivery, because it is non-invasive, is safer and cheaper in comparison to other methods of delivery. Most studies with intranasal gene deposition involved plasmid DNA carrying suppressor, proapoptotic or antiangiogenic genes [4, 14, 21, 49, 50]. Alternatively, gene therapy has exploited siRNA forms designed to limit expression of certain genes important in cancer development mechanisms [41]. The *p53* gene mutated in 50–70% of patients with lung cancer is one of the most frequently targeted genes in experimental projects. Zou et al. [21] have shown that in mice therapy with complexes of poly-L-lysine, protamine and pDNA, encoding for wild type p53 succeeded in induction of significant lung tumor regression and prolonging animal survival. Studies involving another suppressor gene – *PDCD4*, (programmed cell death protein 4) have proven extremely interesting as well. Intranasal administration of pDNA, bearing the *PDCD4* gene, complexed with glucosylated PEI or chitosan, resulted in significant tumor regression due to the increased apoptosis rate of cancer cells and angiogenesis inhibition as well as due to down-regulated expression of genes involved in tumor growth and progression [4, 11]. Another good example of successful studies on the implementation of lung cancer gene therapy into clinical practice is experiments with gene encoding for serin-treonin kinase Akt (protein kinase B), one of the key regulators of cell cycle. Akt1 plays an important role in neoplastic transformation within the respiratory system mostly due to the uncontrolled stimulation of tumor cell proliferation and their apoptosis inhibition. Several animal experimental studies have targeted the Akt pathway either by introducing genes encoding Akt inhibitors or by silencing expression of the *Akt* gene with siRNA [4, 28, 41, 42, 27]. A gene preparation containing pDNA encoding for C-terminal modulator protein CTMP and complexed with a lentivirus vector was evaluated in a K-ras mouse model. It was successfully expressed within respiratory cells after inhalation and triggered Akt1 pathway inhibition *via* selective suppression of Akt1 phosphorylation [27]. In further studies, Hwang et al. [42] confirmed the satisfactory effectiveness of transfection but also the safety and repeatability of intranasal administration. In particular, no time- or dose-dependent reduction in efficiency of gene transfer and expression was observed. Furthermore, prolonged treatment of experimental animals (twice a week for four weeks) resulted in a significant cancer regression observed at different stages of tumor development and accomplished, by appropriate disruption of the cell cycle, the regulation and induction of apoptosis [42].

Inhibition of tumor growth *via* down regulation of the Akt signal pathway has also been demonstrated in a mouse K-*ras*^{LA1} model following administration of pDNA bearing a *Pten* gene complexed with chitosan. *Pten* (phosphatase and tensin homolog deleted on chromosome 10) is a suppressor gene quite often mutated in several malignant tumor types. It was confirmed in the K-*ras*^{LA1} mice expressing a mutated form of *k-ras*, that the inhalation of pDNA bearing a *Pten* gene complexed with chitosan resulted in the effective inhibition of tumor growth and development [4]. Similarly, an aerosolized form of a pDNA coding *Pten* gene complexed with lipofectine administered to B16-F10 (mouse melanoma with metastases to lungs) mouse instigated *Pten* transgene overexpression in the airways followed by diminished metastasis formation in the lungs and prolonged survival time [28]. An siRNA silencing *Akt1* gene inhaled intranasally was also tested in a mouse K-*ras*^{LA1} model, producing approximately an 80% decline in *Akt1* gene expression leading to Akt1 pathway inhibition, modifications within cell cycle regulation, inhibition of tumor cell proliferation and subsequent tumor regression [41].

Interestingly, several groups evaluated the clinical feasibility of combining conventional and gene therapy. Intranasal administration of pDNA encoding for interleukin 12 (IL-12) complexed with PEI administered in combination with intraperitoneal isofosfamid resulted in a significant reduction of the primary tumor mass together with a significantly smaller amount of lung metastasis in mice, acting most probably *via* activation of the Fas/FasL pathway [40]. Gene therapy might also positively support the effectiveness of radiotherapy, as demonstrated by Carpenter et al. [18]. Intranasal administration of liposomes with pDNA encoding for superoxide dysmutase gene (*Mn-Sod*) consistently diminished the occurrence of side effects in mice by inhibiting the activity of oxygen radicals and therefore lung tissue damage. The overall survival of the irradiated animals increased as well [18].

Perspectives

Inhalation gene therapy is a new therapeutic approach to the treatment of patients with cystic

fibrosis, alpha-1-antitrypsin deficiency and lung cancer. Intranasal administration of gene preparations enables not only local transfection of the nasal epithelial cells, but also their effective introduction into the lungs due to the intense perfusion and large absorption area of the mucous membrane. The transfection efficiency of respiratory cells following intranasal gene delivery depends on the vector type and administration route. Research has demonstrated that inhalation of aerosols is much more effective than direct instillation of gene preparations into the nasal cavity. Aerosols allow for non-invasive drug delivery. Preclinical and clinical trials based on virus or non-virus vectors have revealed that gene delivery to the lungs *via* inhalation (instillation) is possible and has fascinating clinical importance. The further development of molecular biology methods and advances in vectorology will cause inhalation gene therapy to become a supplemental or alternative option to the conventional methods of the treatment of lung diseases.

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