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Gene Therapy Perspectives Against Diseases of the Respiratory System

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1. Introduction

Gene therapy uses a variety of techniques as the introduction of a normal allele of a gene in cases where the cell does not express the gene or in other cases where the gene is under-expressed. In order to achieve effective gene therapy for a specific gene in a certain type of cells a lot of work is needed. More specifically the following steps are essential: 1. Isolation of target gene, 2. Development of a specific gene vector, 3. Specification of the target cell, 4. Definition of route of administration, and 5. Identification of other potential uses of the gene.

The value of gene of gene therapy is often discussed, especially in some diseases who have a known protein defect and the protein itself can be produced in a large scale and could then be administered to the patient. Genetic engineering could be beneficial in the production of the target protein. Nevertheless, the infusion of the protein is not curative, because of the half-life of the protein itself and the growth factors that are essential.

In order to isolate a specific gene, it is essential to produce a cDNA library that contains the total number of unique genes expressed in a specific tissue. The DNA contained in a cDNA library is not genomic, therefore it contains only the encoding sequences of the DNA.

The standard procedure of the construction of a cDNA library includes 1) isolation of the total amount of mRNA that is produced in the target cells, 2) Hybridization using a multi-T promoter, 3) Synthesis of complementary DNA (cDNA) to the mRNA prototype using the enzyme reverse transcriptase, 4) Degradation of the mRNA by the means of an alkali, 5) Synthesis of the second DNA strand using nucleotides and the enzyme DNA polymerase.

The cDNA library contains only the exons of the genes that are expressed in the specific tissue; therefore the cDNA can show the activity of the studied tissue.

As soon as the isolation of the gene that is to be administered to the patient is achieved, an appropriate vector is needed in order to deliver the gene to the target cells. The most important vectors that are generally used in gene therapy applications in order to perform transfection of the targeted cellular population are:

1. Plasmids which are well-tolerated and safe, but transfer towards the nucleus is not so easy

2. Adeno-virus which may transfect differentiating as well as stale cells and have a very good percentage of transfection, but is not inserted in the nucleus and there is a possibility of reaction against the adeno-virus

3. Retro-virus which are inserted in the genome and are stable during transport, but they can only used in transfection of multiplying cells

4. Lenti-virus which is a subtype of retro-virus that may be inserted in stable cells and it is quite stable during the procedure

5. AAV (adeno-associated-based vector) which is inserted in the genome, is quite stable during the procedure and stable cells can be transfected as well, but only 4,7 kb can be inserted and there is a possibility of mutations

6. Liposomes – Oligonucleotides (ODN-based) which are very easy to use, selective for the endothelium, special alterations can improve the availability and reduce toxicity

The target cell has to be defined carefully in order to achieve the best curative result. In the case of gene therapy in the lung, the airway epithelia or even the lung vasculature may be efficient cellular targets.

The route of administration has to be defined so as the target gene is transported to the target cells in order to perform the transfection of the target tissue cells.

The use of a target gene in the therapy of a certain condition of the lung does not exclude a possible use of the gene in another therapeutic strategy, where there is a similar pathophysiology (e.g. inflammation). Therefore, the identification of other potential uses of the target gene is always important.

2. Gene therapy in cystic fibrosis

Gene therapy is still far from becoming a curative treatment for cystic fibrosis (CF). Despite the outstanding technological and medical progress there is still number of interesting genetic, biological, pharmaceutical and ethical problems. Only when these issues are to be solved will gene therapy become an option for the treatment of CF.

As for the biology of CF, the cystic fibrosis transmembrane conductance regulator (CFTR) is expressed in airway epithelia, on the luminal side of the plasma membrane, where it plays

an important role as a phosphorylation-regulated Cl− channel and a regulator of channels and transporters [1, 2]. More specifically, the activation of CFTR results in a parallel inhibition of the epithelial Na+ channel (ENaC), which is lost when CFTR is absent or not functioning. There is a so called "low volume" hypothesis, which suggests that a loss of Cl− secretion and an increase in Na+ absorption reduce the thickness of the airway surface liquid (ASL), thus impairing mucociliary clearance [3]. Moreover, a reduction in the secretion of bicarbonates (mediated by the CFTR) might affect the hydration of the secreted mucus, thus altering its physical properties [4]. CFTR is also expressed in submucosal glands in the airways, which mainly participate in host defence. A loss of CFTR function in duct-lining serous cells prevents the secretion of mucus and anti-microbial factors by submucosal glands [5].

Since the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in 1989, it was thought that scientists could prevent or delay the onset or even the progression of lung disease by using gene transfer. Although loss of CFTR function may affect a great number of different cells and tissues, progressive lung disease is responsible for the rates of morbidity and mortality. Therefore, the efforts of gene therapy have focused so far on gene transfer to the airways. CFTR is expressed in various epithelial cells in the lumen and in submucosal glands of the airways, where the mRNA is expressed [6, 7].

The fact that CF is an autosomal recessive disease lead to the idea that the delivery of a CFTR cDNA to the airway epithelium with a viral or non-viral vector could have beneficial effect. The delivery method could be either direct instillation or aerosol delivery. Furthermore, early studies indicated that the transfection of 6–10% of CF epithelia generated wild-type levels of chloride transport in vitro [8].

The selection of targets cells for gene therapy in CF is still controversial. The available strategies suggest correcting cells of the surface epithelium, the submucosal glands, or both [9, 10, 11]. The CFTR is expressed in the airways, including ciliated cells within the surface epithelium and a subpopulation of cells in submucosal gland ducts and acini. There are several epithelial cell types in the lung that seem to have progenitor functions, thus allowing long-term correction if these cells are targeted with selected vectors [12]. Experiments from several species and model systems have revealed potential progenitor populations, including: basal cells [13] and non-ciliated columnar cells of the airways [14, 15], submucosal gland epithelia [16], Clara cells [17] and alveolar type II cells in the distal lung.

Many viral and nonviral vectors have been tested for their usefulness in CF gene therapy. Adenoviral (AV) vectors have as a great disadvantage their low transduction efficiency of human airway epithelia and by their induction of strong immune responses [18]. In contrast adeno-associated viral (AAV) vectors may lead to long-term gene transfer and expression in bronchial epithelia of rabbits and nonhuman primates.

In addition to the DNA viruses, AV and AAV, various RNA viruses have been investigated for uses in airway gene transfer. Murine parainfluenza virus type 1, human respiratory syncytial virus (RSV) and human parainfluenza virus type 3 (PIV3) can effectively transfect airway epithelial cells by attaching to sialic acid and cholesterol [19], which are found on the

apical surface of these cells. These viruses replicate in the cytoplasm and do not seem to cause mutagenesis during the insertion in DNA. Although RSV and PIV3 are human pathogens, SeV, the only RNA virus for which efficiency has been assessed in vivo, is not. However, gene expression mediated by recombinant SeV-based vectors needs repeated administration, which does not seem feasible because of the development of neutralizing antibodies against the vector itself [20].

Lentiviral (LV) vectors derived from human immunodeficiency virus type 1 (HIV-1) and feline immunodeficiency virus (FIV) are integrating retroviruses which can be adequately utilized to achieve efficient transfection of airway epithelia [21].

Among the many nonviral gene therapy vectors investigated so far, GL67 ([Cholest-5-en-3-ol(3b)- 3-[(3-aminopropyl)[4-[(3-aminopropyl)amino]butyl] carbamate]) has emerged as a promising lipid for efficient lung transfection [22].

Finally, mRNA-based nonviral gene transfer is a new strategy in order to express the CFTR in target cells [23]. By the use of mRNA instead of plasmid DNA as the transgene, transfection efficacy depends on the cytoplasmic expression machinery. However, when compared to DNA, much less is known about immune responses to RNA, although responses to both seem to be mediated by Toll-like receptors (TLRs).

As a chronic, lifelong disease, CF will be best treated with a continuous level of CFTR expression. This could be achieved either by repeated application or with a long-duration expression system. Viral vectors, which are mainly used in gene therapy appear difficult to administer repeatedly [24], in contrast to synthetic approaches [25].

The use of genomic DNA that contains all the control elements that allow gene expression at physiological levels has been utilized [26]. Extensive knowledge of the critical regulatory elements in the CFTR locus is required.

The CFTR gene maps at 7q31.2 and the expression is regulated during development and in different tissues. The CFTR locus is in connection with genes with different tissue-specific expression profiles, suggesting the presence of specific control promoters and insulators. Nuclear localization studies of CFTR and its adjacent gene loci in humans and mice demonstrate that different chromatin regions behave independently, depending on their expression profiles [27].

2.1. Applications of RNA interference to treat CF

The recent knowledge in the field of small interfering RNAs has led to the development of applications in relevance to CF. The RNAi technology has been used in order to identify gene products that contribute to steps in wild-type and mutant CFTR production and action [28]. Therefore, there is a possibility that RNAi-based strategies could be developed to increase the expression of ∆F508 CFTR, to rescue ∆F508 CFTR from proteosomal degradation or prolong its action on cell membrane. Similarly, targeting other cellular pathways, such as the inflammatory process, might lead to the reduction of symptoms. A significant obstacle

to overcome is the identification of methods to efficiently deliver RNAi to differentiated airway epithelia.

2.2. Lung tissue engineering

Lung transplantation is currently the only definitive treatment for end-stage CF lung disease. However, the availability of donors is limited and the survival of transplantation is hardly 10–20% at 10 years [29]. Recently, two groups independently used similar tissue-engineering strategies to develop an autologous bioartificial lung that may begin to help overcome the limited availability of donor tissues [30, 31]. Evidence for gas exchange within the resulting grafts was demonstrated. Following the development of this technology, the ex vivo correction of patient-derived cells and the transplantation of these cells could lead to the cure of the disease. Although these initial results are very exciting, several steps need to be further optimized before long-term tissue-engineered lung function can be used in patient applications [32].

3. Gene therapy in Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease is a disease characterised by the presence of airflow obstruction generally progressive due to chronic bronchitis or emphysema and may be partially reversible. COPD is the 4th leading cause of death in the United States. In 2000, the WHO estimated 2.74 million deaths worldwide from COPD. In-patient hospitalization and emergency department care accounts for >73% of this cost COPD costs $1,522 per person per year (3 times asthma costs) [GOLD 2008].

Tobacco smoke is by far the most important risk factor for COPD worldwide. Other important risk factors are occupational exposure, socioeconomic status and genetic predisposition [33]. Thus, investigating into copd and into management possibilities is of high importance in order to provide the essential help to the patients. The currently used drugs can manage effectively the main symptoms of copd and may control the symptoms of this condition.

To date, there are no effective treatments for emphysema, nor are there efficient clinical management strategies. Novel approaches using gene therapy and stem cell technologies may offer new opportunities. However, this will remain almost entirely dependent on a more thorough understanding of the pathogenesis of COPD [34]. Currently, the most accepted theory for the development of COPD is protease/ antiprotease imbalance similar to emphysema due to hereditary 1-antitrypsin deficiency [35]. Newer studies [36] have shown that the pathogenesis of COPD involves not only elastases, but also collagenases and gelatinases. Experimental models [37] have suggested a role for 1-antitrypsin and secretory leukoprotease inhibitor in the treatment of this disorder. However, there is still need for a convincing study proving the concept of antiprotease treatment for COPD and emphysema [38] Neutrophils are a major source of proteases and reactive oxygen, so gene therapy could also target adhesion molecules for neutrophils to reduce their accumulation into the lung parenchyma.

3.1. A1-antitrypsin deficiency

A1-antitrypsin (AAT), is a major anti-protease serum protein, counteracting the effects of neutrophil elastase and other pro-inflammatory molecules released at sites of lung inflammation [39]. There are not effective treatments using protein therapy so gene therapy is being evaluated as an alternative approach.

Early studies in cotton rats using first-generation Ad vectors resulted in detection of AAT in bronchoalveolar fluid for only 1 week post-administration [40]. Cationic liposomes have also been used to express human AAT in the rabbit lung following aerosolisation [41]. Recombinant AAV vectors are being evaluated for more persistent expression of therapeutic serum levels of human AAT in murine and non-human primate models following intramuscular injection [42]. A1-antitrypsin deficiency is a pulmonary disease with an underlying single gene defect and a target for gene therapy. One specific treatment for AAT deficiency available is the administration of AAT intravenously, but only 2–3% of the infused AAT actually reach the lungs. Another method of administration is the inhalation of nebulized AAT powder or aerosolized AAT solution [43]. However, the treatment by the means of an alternative therapy, namely gene therapy, provides long term solution [44]. Several vectors containing cDNA of AAT have been constructed for treating AAT deficiency diseases. These vectors are retroviral [45], adenoviral [46] and adeno-associated viral [47]. Besides this, AAT gene can also be transferred by liposomal vectors [48]. First clinical trial has demonstrated that AAT gene could be transferred in humans [49]. Patients with AAT deficiency received a single dose of non-viral cationic liposome. Protein Gene Therapy for Alpha-1-Antitrypsin Deficiency Diseases was detected in nasal lavage fluid, with maximum levels on fifth day, which is approximately one third of the normal levels. The retroviral vector containing cDNA of human AAT with constitutive promoter have also been used as a delivery system. The disadvantage of retroviral vector system is that transgene expression is low. The adenoviral vectors containing human AAT cDNA have been delivered to different organs and cells [50]. Results in vitro demonstrated that human alpha-1-antitrypsin was synthesized as well as secreted. The adenoviruses are pathogenic in nature as well as immunogenic, therefore they have limited applications in treating AAT deficiency diseases. Recombinant adeno-associated viral vectors have been most successful delivery system so far, as they are capable of achieving therapeutic levels of AAT [51], and are less likely to induce an inflammatory response than adenoviral vectors. These viral and non-viral vectors showed advantages as well as disadvantages in curing AAT deficiency diseases. Among tested rAAV serotypes, the rAAV8 was found to be more powerful gene therapy vector for treating lungs and liver diseases [52]. Newly developed AAV vector looks promising for treating AAT deficiency diseases.

4. Gene therapy in asthma

Asthma is a disorder defined by certain clinical, physiological and pathological characteristics. Asthma is a chronic inflammatory disorder of the airways associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest

tightness, and coughing, usually associated with widespread, but variable, airflow obstruction that is often reversible either spontaneously or with treatment [GINA 2000]. Since its pathogenesis is not clear, this definition is descriptive and inclusive of different phenotypes that are being increasingly recognized. Worldwide, 300 million people are supposed to be affected by asthma [53]. It appears that the global prevalence of asthma ranges 1–18% of the population in different countries. The WHO has estimated that 15 million disability-adjusted life-yrs are lost annually due to asthma, representing 1% of the total global disease burden [54]. Annual worldwide deaths from asthma have been estimated at 250,000 and mortality does not appear to correlate well with prevalence.

The best treatment of asthma is inhaled corticosteroids and bronchodilators, for the majority of asthmatic patients [55]. Gene therapy could bring some benefit for asthmatic patients with uncontrolled asthma who require high doses of corticosteroids and for patients with corticosteroid- resistant asthma. The target of gene therapy in bronchial asthma could be the overexpression of T-helper (Th) type 1 cytokines that influence the Th2 cytokine reactions [56]. Moreover the overexpression of IL-12 also restored local antiviral immunity, which is impaired in a Th2-dominated environment particulary during exacerbations of bronchial asthma due to viral infections [57]. Another study [58],examined the gene transfer of IFN that is a very interesting mediator in the airway hyper-responsiveness. Furthermore, another newer study [59] has shown that the transfer of the glucocorticoid receptor gene in vitro mediated the inhibition of nuclear factor-B activities even in absence of exogenous corticosteroids, and the authors suggested that this approach could restore corticosteroid sensitivity in patients.

5. Gene therapy in lung cancer

Lung cancer is the most common cancer worldwide, it is responsible for 12.4% of new cases of cancer in 2002. The overall mortality is 87% and 5-year survival is estimated to range from 15% in USA to 8.9% in developing countries [60]. It ranks first as the cause of death and it is responsible for 1.18 million deaths in 2002 and it is accounted by the World Health Organization for 18.4% of all cancer deaths by 2015. Non-small cell cancer (NSCLC) accounts for approximately 85% of lung cancers [61].

Even if there have been a lot of advances in surgery, radiation and chemotherapy, the 5-year survival for lung cancer remains poor. There is now great interest in gene therapy approaches for thoracic malignancies. Lung cancer is usually metastatic at the time of diagnosis and systemic therapy is needed rather than local therapy.

Gene therapy for thoracic malignancies represents a therapeutic approach that has been evaluated in clinical trials for the last two decades. Using viral vectors or antisense RNA, strategies have included induction of apoptosis, suicide gene expression,cytokine based therapy, various vaccinations and adoptive transfer of modified immune cells.

5.1. Clinical trials

5.1.1. Replacement of tumor suppressors

The goal of this strategy is to use a gene vector in order to encode a tumor-suppressor protein in tumor cells that is mutated or absent in the majority of lung cancers.

Tumor-based p53 therapy: It has been shown that the replacement of the normal p53 tumor suppressor gene in tumor cells induces rapid cell death by studies in cellular and animal models. In several early-phase clinical studies the strategy of the restoring the wild-type p53 expression in lung tumor cells was studied. The first study to demonstrate the feasibility of tumor suppressor gene replacement mediating tumor regression was a phase I study in which a retrovirus vector carrying wild-type p53 was administered to 7 patients with lung cancer with direct intra-tumoral injection. There was evidence of increased apoptosis in 6 patients and tumor regression in 3 patients [62]. Other phase I studies of p53 replacement with adenoviral vectors resulted in few partial responses and several patients with stabilization of disease[63, 64, 65]. Weill et al. delivered Ad.p53 to obstructive lesions endobronchially and they had several partial responses [66]. In another large phase I study of Ad.p53 gene transfer that was delivered intra-tumoraly in combination with chemotherapy, it was shown that there was increased apoptosis in transduced tumors when examined histologically [67]. A single-arm phase II study of intratumoral Ad.p53 in combination with radiation showed tumor regression in 63% (12 of 19) and was well tolerated [68]. But in another phase II study there was no difference in response rates for Ad.p53/chemotherapy-treated lesions for primary tumor lesions versus lesions treated with chemotherapy alone and this showed that Ad.p53 provided little local benefit over chemotherapy [69]. Keedy et al. delivered repeatedly Ad.p53 by bronchoalveolar lavage (BAL) to patients with bronchoalveolar carcinoma. It was shown that this delivery resulted in transient expression of p53 in 19% (3 of 16) of patients,2 of the 3 patients achieved stable disease. It was suggested that BAL could be used for adenoviral delivery, but toxicity was a serious issue with this approach [70]. Guan et al. delivered Ad.p53 alone or in combination with bronchial artery instillation (BIA) of chemotherapy (fluorouracil, navelbine or cisplatin). The delivery of Ad.p53 was performed via direct percutaneous delivery or via BIA. There was 47% response rate in the combination group and an improvement in time to progression when compared with BAI alone [71]. The Adp53 has been approved for usage in neck and head cancers in China, but there are not any trials using Ad.p53 in lung cancer in USA. Vanchani et al. believed that there is a strong issue with the application of this method in lung cancer (especially treating endobronchial lesions) as there is no bystander effect in combination with low transfection efficiency of adenoviral vectors [72].

FUS1 Replacement: FUS1 is a novel tumor suppressor gene that was identified in human chromosome 3p21.3 region where allele losses and genetic alterations occur for some human cancers. In most premalignant lung lesions and lung cancers the expression of FUS1 protein is absent. It was shown that wt-FUS1 function was restored in 3p21.3-deficient non-small cell lung carcinoma cells and this function inhibited tumor cell growth by induction of apoptosis and alteration of cell cycle kinetics [73].

Gene-Modified Dentritic Cell-Based Vaccination: Dentritic cells (DC) are the most potent antigen presenting cells in the immune system and they have been used for vaccination as vaccine vehicles. They have been used in two ways. The first one is to modify DC ex vivo with chemokines or cytokines and inject them directly into tumors and then they take antigen and induce immune response. The second one is to load immature, phagocytic DC with antigen with the aid of purified protein, cell extracts, mRNA and gene vectors and after that they inject these DC subcutaneously.

Ad.p53: p53 protein: It is proposed as a tumor antigen for vaccines as mutant p53 exists in very high levels in tumor cells and has more prolonged half-time than normal cells. p53-based gene therapy (p53 transduced DC) with standard chemotherapy showed promising results [74]. In a phase I trial, 29 patients with small cell lung cancer were vaccinated with DC transduced with Ad.p53 and the result was 1 patient with partial response and 7 cases with stable disease. Besides, out of the 21 patients that received a second line of chemotherapy, there was 62% response rate much higher from the rate that it is known for the second line therapy in small cell lung cancer. There was also a better survival (12.1 months instead of 9.6 months) in patients that showed an immune response to vaccination.

CCL21: CCL21 is a CC chemokine which is expressed in high levels in high endothelial venules and T cell zones of spleen and lymph nodes and also it attracts mature DC, naive T cells and induces T-cell activation [75]. Preclinical data showed that there was potent activity against lung cancers when DC transduced with CCL1 were injected into tumors.

Gene-Modified Tumor Cell-Based Vaccination: Killed tumor cells (usually irradiated) have been injected into patients as vaccines against recurrent cancers for many years with partial successful results.

Transforming growth factor β2 antisense vector modified cells: It is known that increased levels of transforming growth factor (TGF-β2) are associated with greater immunosuppression and poorer prognosis in patients with NSCLC. Preclinical studies showed that the delivery of an antisense gene to TGF-β2 to ex vivo tumor cells inhibited cellular TGF-β2 expression and resulted in increased immunogenicity when these tumor cells were administered as a vaccine. In a phase II trial this method of vaccination with irradiated tumor cells modified with a TGF-β2 antisense vector (belagenpumatucel-L) was evaluated. There was better survival (dose-related) with minimal toxicity. Besides there were different immunologic end points such as increased levels of cytokines (INF-γ, interleukin-6,interleukin-4) and increased levels of antibody production to vaccine HLAs. In a trial, 21 patients received belagenpumatucel-L at a single dose [76]. It was shown that 70% of cases were stable, but there was no complete or partial response. There is an ongoing phase III trial in which this vaccination is evaluated.

Tumor cells modified to secrete granulocyte-monocyte colony stimulating factor(GVAX): Granulocyte-monocyte colony stimulating factor (GM-CSF) is a cytokine that is involved in the maturation and proliferation of myeloid progenitor cells and stimulates proliferation, maturation and migration of DC and that leads to induction of T-cell immune responses against cancer. There are preclinical studies in which the transfection of tumor cells with the

GM-CSF gene has led these cells to induce antitumor immune responses. The clinical trials in lung cancer started using a vaccine platform with intradermal vaccination of irradiated autologous tumor cells that were virally enginnered to secrete GM-CSF [77, 78]. In the first trial of cases of metastatic NSCLC, GM-CSF was transduced into autologous tumor cells with the aid of adenoviral vector before irradiation and vaccination. There were a few clinical responses with a strong immune response. A delayed hypersensitivity reaction to irradiated, autologous nontransfected tumor cells was observed in patients. Nemunaitis J,et al. used a similar strategy in early-stage and late-stage patients and they showed that there were several clinical responses with similar immunologic outcomes [79]. In another trial, Nemunaitis J,et al. used a vaccine of unmodified, irradiated autologous tumor cells mixed with a GM-CSF-secreting bystander cell line. The vaccine GM-CSF secretion was higher than with the autologous vaccine, but the frequency of vaccine site reactions, tumor responses and survival were less favorable with the bystander vaccine [80]. Finally, the GVAX approach was not used more in lung cancer because the results were not satisfied and now only studies in pancreatic cancer are going on.

a(1,3)Galactosyltransferase: The gene that encodes a(1,3) Galactosyltransferase is not active in humans and it is functional in other mammalian cells. The major mechanism of hyperacute rejection of xenotransplants is the production of anti-a Gal antibodies in humans. Morris J et al. used allogeneic NSCLC tumor cells that were retrovirally modified to express aGT. It was shown that 6 of 17 patients, that received intradermal treatments, had prolonged stable disease [81].

B7.1/HLA vaccination: B7.1 is the one that costimulates T cells during priming by an antigen-priming cell. In a phase I trial of 19 patients with advanced NSCLC, treatment with an allogeneic lung cancer cell line vaccine transfected with B7.1,HLA-A1 and HLA-A2 was done. There was one partial response and 5 cases with stable disease. In the 6 responders, the CD8 T cell titers to tumor cell stimulations were elevated steadily till 150 weeks after therapy [82].A phase II trial is ongoing in patients with stage IIIB/IV who fail after the first line chemotherapy.

5.1.2. Vaccines

MUC-1 vaccination: MUC-1 is a tumor-associated mucin-type surface antigen normally found on epithelial cells in many tissues. In cases of lung cancer the targeting of MUC-1 has been used in a lot of ways with gene and non-gene therapy approaches. Ramlau R et al. in their 2 arm phase II trial with 65 patients with IIIB/IV NSCLC used a vaccinia virus containing the coding sequences for MUC1 and IL-2 (TG4010).The patients that participated in the trial had MUC-1 antigen expression on the primary tumor or metastases. In the 1ST arm (44 patients), combination therapy with TG4010 and cisplatin/vinorelbine was given, and in the 2nd arm TG4010 monotherapy was given followed by combination therapy at progression. In the 1st arm there was partial response in 29.5% and survival rate of 53% for the 1st year. In the 2nd arm, two of the 21 patients had stable disease for more than 6 months with monotherapy of TG4010 and this arm was terminated early as the results were not satisfied. There were MUC1-specific responses for 12 of 21 patients with stable disease or partial response.

Disease control was observed for 4 of 5 patients. The existence of MUC1 specific responses was translated to longer time to progression and better overall survival [83].

L523S vaccination: L523S is an immunogetic lung antigen that is expressed in 80% of lung cancer cells. Nemunaitis et al. in a phase I study, they gave two doses of intramuscular recombinant DNA followed by two doses of Ad.L523S (given 4 weeks apart) to 13 patients with early stage NSCLC(stage 1B,IIA and IIB). The authors found that only 1 patient showed a L523S-specific antibody response [84].

5.2. Antisense therapy

This technology is able to downregulate a lot of molecules that promote lung cancer tumor growth. There are 3 trials with antisense therapy. In the first trial aprinocarsen was used. Aprinocarsen is an oligonucleotide that binds to mRNA for protein kinase C-a and inhibits its expression. It was demonstrated that this molecule was safe in patients with lung cancer and it was characterized by modest activity in combination with chemotherapy [85]. In another trial with chemotherapy with or without aprinocarsen as first line therapy, it was shown that there was no better survival but with some toxicity as well [86]. In phase I studies it was shown that few patients had prolonged stable disease and 1 patient had response with the administration of Raf antisense molecules [87]. In patients with lung cancer in two phase II studies, these molecules did not show any antitumor activity [88]. Next, in other trials, the authors used Bcl-2, an apoptotic inhibitor which is overexpressed by many tumors and especially by 80-90% of SCLCs; the existence of this inhibitor means increased resistance to chemotherapy. In two trials there were encouraging results [89, 90], but in another trial of standard chemotherapy with or without a bcl-2 antisense oligonucleotide (oblimersen) more hematologic toxicity and worse overall survival was observed in the experimental arm [91].

5.3. New directions

The trials that have been made about gene therapy in lung cancer, preclinically and clinically, have demonstrated intermittent efficacy. The technology of gene transfer is promising but it is not easy to transduce more than a small number of tumor cells. This is a very important issue especially with approaches that they do not have bystander effects. It is very important to create vectors that they are able to induce long term in vivo expression as lentiviruses and AAVs.

Another interesting strategy is the immune-gene therapy, which requires gene transduction for stimulating an endogenous immune response and in this way a bystander effect is generated. There are some engouraging approaches with gene therapy to stimulate anti-tumor responses by delivering immunostimulatory cytokines or by administering a vaccine.

There is another important field of creating adoptive transfer of gene-modified autologous lymphocytes that are modified ex vivo by using lentiviruses or retroviruses. This approach is directed against mesothelioma and lung cancer cells.

6. Conclusion

Gene therapy is a very promising tool for the respiratory clinician and a few clinical trials have been performed. All these trials have shown safety but intermittent efficacy. Gene therapy for pulmonary diseases has not yet reached the point of clinical practice. But we can say that this tool will find a very interesting role in our efforts for treating respiratory diseases in the future.

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