

Gene therapy in management of lung cancer

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Lung cancer is the most common cause of death, related to malignant disease. Both major types - small cell and non-small cell lung cancer (SCLC and NSCLC, respectively) possess properties, which significantly affect the survival rates of onco-patients. During the treatment, there occur several issues, including development of chemoresistance, fast metastatic expansion or inoperability. Although, there are only few examples of how gene therapy can be helpful in combating the cancer, its potential is undisputed. Presented study describes and discusses the current status and possibilities of gene therapy, including the viral and non-viral vectors, antisense strategy of gene silencing and the latest technology using CRISPR-Cas9 in treatment of lung cancer.

Keywords: Antisense; CRISPR-Cas9; Chemoresistance; NSCLC; SCLC; Vectors

1. Introduction

Lung cancer is the most common cause of cancer-related deaths worldwide. Despite advances in diagnostics and therapeutics of lung cancer, a 5-year survival rate is still reaching only about 15% [1]. This disease largely affects the socioeconomic statuses of patients and their families, as well as the society. Clinical and molecular evidence has proven that lung cancer is a heterogeneous disease, which demonstrates significant implications in diagnosis [2] and treatment [3]. An increasing number of clinical trials have emphasized targeted and personalized treatments that specifically benefit patients diagnosed by using observed gene expression profiles. The term lung cancer usually refers to tumors that originate from the lining cells of the respiratory tract (epithelial cells) [4]. Based on differences in biological characteristics, lung cancer is classified into two types, namely non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts approximately 85% of lung cancer cases [5]. Platinum-based chemotherapy is prescribed

as the standard first-line therapy in patients with advanced NSCLC. However, resistance to platinum-based drugs reduces the survival rate which, as a result, has not improved to anything like the extent seen in other cancers [6]. Advances in the understanding of molecular genetics in NSCLC have led to the identification of key genetic aberrations in NSCLC. These genetic aberrations occur in oncogenes that encode signalling proteins that are crucial for cellular proliferation and survival [7]. Genetic profiling has identified driver mutations in over 60% of lung adenocarcinomas, with 9–14% being new targetable oncogenes such as HER2, BRAF, PIK3CA, and RET [8]. SCLC accounts 10–15% of all lung cancer cases and represents the most aggressive subset of lung cancer. Treatment of SCLC has changed minimally over the last few decades. Patients continue to receive non-targeted, chemotherapy regimens consisting of etoposide plus platinum agents, often combined with irradiation. Although SCLC patients respond well to this first-line treatment, relapse is virtually inevitable and resultant tumours are resistant to further therapy [9]. Based on above

mentioned information, in lung carcinoma management, the personalized medicine is at the forefront, with the goal to cure patients with a predicted modality to be efficacious based on the molecular characteristics of the tumor. Such approach can offer increase of survival rates of oncopatients, significant reduction of tumor chemoresistance and decrease of a number of potential relapses. Gene therapy can be a powerful weapon to combat lung cancer and to elevate the therapeutic successes.

2. Gene therapy

Gene therapy can be defined as the transfer of genetic material into a cell for therapeutic purposes [10]. Gene therapy was conceptualized in 1972, by authors who urged caution before commencing human gene therapy studies. The first gene therapy experiment approved by the US Food and Drug Administration occurred in 1990, when Ashanti DeSilva was treated for adenosine deaminase deficiency with severe combined immunodeficiency. By January 2014, some 2000 clinical trials had been conducted or approved and no one was FDA approved for clinical utilization in management of lung cancer. Despite these facts, gene therapy still offers huge potential, which nevertheless encounters numerous obstacles. The aim of present study is to summarize the approaches employable for gene therapy of lung cancer and to highlight their possible advantages and disadvantages. Given the large size and the negative charge of these macromolecules, their delivery is typically mediated by carriers or vectors as is discussed below.

2.1 Gene delivery

Gene therapy relies on the principle of introducing exogenous DNA into malignant cells causing them to die. Since lung cancer can be a highly disseminated malignancy, the gene therapeutic agent must be administered systemically, obligating a high level of targeting of tumor tissue and the use of delivery vehicles designed for systemic circulation of the therapeutic DNA [11]. The possible target cells include not only the tumor cells and the immune cells but also surrounding normal tissue. Gene therapy of

tumor cells could result in correction of their abnormal growth and re-establishment of apoptosis, or in increased drug or radio-sensitivity of the tumor cells. Gene modification of tumor cells could also enhance their immunogenicity [12]. Various physical and biological methods are available to deliver genes into target cells. Which delivery method one chooses depends generally on the local, regional, or systemic route of administration chosen or needed to reach the tumor [13]. Physical methods, such as calcium phosphate precipitation, electroporation, direct microinjection, and gene gun, may be suitable for introducing naked DNA into established cell lines *in vitro*, but are generally of low efficiency and are often impractical for *in vivo* applications. Nevertheless, gene delivery to lung tumors by aerosolization of adenoviral vectors incorporated into calcium phosphate precipitates resulted in much greater expression in tumors than in normal lung tissue [14]. Biological vectors - genetically modified, replication-defective viruses are effective by exploiting their natural tropism for mammalian cells and biological life cycles to achieve gene transfer and gene expression. Retroviral vectors can infect a variety of cell types and have the advantage of being able to integrate into the target cell genome. However, because retroviral-mediated transduction might result in permanent integration of the foreign gene into the target cell, the promoter used to drive the transcription of the foreign gene must be carefully selected. Moreover, one of the biggest challenges facing viral vectors in gene delivery is the host immune response. Cell-mediated responses to viral vectors have been documented, but this response may be dependent on the route of administration [15] and vector serotype [16]. For instance, a potent immune response to adeno-associated virus-ovalbumin was observed when vector was administered intraperitoneally, intravenously, or subcutaneously but not when administered intramuscularly [17]. A replication-deficient type 5 adenovirus (Adp53) in which the viral E1 gene was replaced with a wt p53 expression cassette driven by cytomegalovirus promoter has been evaluated in two Phase I clinical trials in NSCLC patients [18,19]. From these trials, it

was found that disease stabilization lasted up to 14 months, and >50% tumor regression was seen in two patients. The observed response rate in this heavily pretreated group of patients with progressive disease was encouraging.

Non-viral gene delivery strategies are generally regarded as safer and less immunogenic alternatives to viral vectors. Nonviral methods of gene delivery have recently expanded and several effective nanomaterials exist including lipid-based [20], polymeric [21], and inorganic nanoparticles [22], some of which have reached clinical trials. Modern non-virals vector are characterized by a high level of transfection efficiency, low production costs, they are easy to prepare and enable a flexible size of DNA to be transported. As was shown by Ji and colleagues, restoration of wt-FUS function in 3p21.3-deficient NSCLC cells significantly inhibits tumor cell growth by induction of apoptosis and alteration of cell cycle kinetics [23]. A Phase I clinical trial is underway to evaluate delivery of the FUS1 gene using repeated intravenous injection of liposomal particles composed of DOTAP and cholesterol.

2.2 Antisense oligonucleotides (ASOs)

The field of oligonucleotides (ODNs) has been developed in a sophisticated manner and novel pharmaceuticals appear to emerge based on ODNs. Evidently, several approaches using ODNs could be done at the gene therapy level using the ODN genes. This will reduce cost, toxicity and ensure the presence of steady state levels of a therapeutic ODN in the cytoplasm or nucleus [24]. Because nucleases that cleave the phosphodiester linkage in DNA are expressed in almost every cell, unmodified DNA molecules are generally degraded before they reach their targets. Therefore, antisense drug candidate molecules are generally modified during the drug discovery phase of their development [25]. Amongst the most successful nucleic acid backbone modifications belong phosphorothioates, morpholinos, locked nucleic acids (LNAs), ribozymes or peptide nucleic acids (PNAs) [26-28]. In lung cancer management, the rational drug design has resulted in agents directed against a number of important cellular

targets, including the mRNA of bcl-2, cyclin D1, protein kinase (PK) C-alpha, PKA-I, H-ras, c-raf, R1 and R2 subunit of ribonucleotide reductase, and transforming growth factor beta2 [29,30]. Saini and Klein demonstrated that NSCLC and mesothelioma cells can be significantly weakened by using CD1 ASOs within the meaning of cell proliferation and CD1 de novo synthesis [30]. Other suitable targets are cyclooxygenases. For instance cyclooxygenase 2 (COX-2), overexpressed in several tumor entities, can be efficiently blocked, as was shown by Windhovel and coworkers [31]. Interestingly, they tested twelve phosphorothioates and a range of activities was reached on protein, RNA and growth level. This points at importance of selection of a duplexing site on mRNA sequence, which subsequently affects the translation into protein.

2.3 CRISPR-Cas9

CRISPR-Cas9 is a versatile genome editing technology for studying the functions of genetic elements. The bacterial type II clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) systems have recently shown great potential for RNA-guided genome editing, including multiplexing genome engineering, homologous recombination, gene targeting and regulation of transcription [32]. Using both, viral (lentivirus) and non-viral (nanoparticles) mediated delivery of guide RNA, Platt et al. simultaneously modeled the dynamics of KRAS, p53, and LKB1, the top three significantly mutated genes in lung adenocarcinoma [33]. CRISPR-Cas9 technology arises many discussions in the scientific community, particularly due to its exceptional properties, thus it can be expected that CRISPR-Cas9 can significantly affect future of modern medicine, not only within the meaning of malignant diseases.

3. Conclusion

Although limitations still exist to the widespread application of gene therapy, the strategy has been shown to be applicable in several clinical situations. Currently, the most fundamental issue is development of efficient and non-immunogenic vectors for delivery of

nucleic acids, which are easily cleaved by endonucleases. Another option is further development of nucleic acids backbone modifications, which provide higher stability and are nucleases-proof. Gene therapy offers a number of future possibilities from simple blocking of expression through mRNA duplexing to highly effective replacement and deletions of entire dysfunctional genes. Hence, gene therapy can be considered as powerful future-weapon to combat lung cancer.

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Conflicts of Interest

The authors declare no conflict of interest.

References

- Siegel, R.; Ma, J.M.; Zou, Z.H.; Jemal, A., *Cancer Statistics, 2014. Ca-a Cancer Journal for Clinicians* 2014, 64, 9-29.
- Cha, N.; Lv, M.; Zhao, Y.J.; Yang, D.; Wang, E.H.; Wu, G.P., *Diagnostic utility of VEGF mRNA and SP1 mRNA expression in bronchial cells of patients with lung cancer. Respirology* 2014, 19, 544-548.
- Xu, R.; Shang, C.; Zhao, J.G.; Han, Y.; Liu, J.; Chen, K.B.; Shi, W.J., *Knockdown of response gene to complement 32 (RGC32) induces apoptosis and inhibits cell growth, migration, and invasion in human lung cancer cells. Molecular and Cellular Biochemistry* 2014, 394, 109-118.
- D'Adda, T.; Pelosi, G.; Lagrasta, C.; Azzoni, C.; Bottarelli, L.; Pizzi, S.; Troisi, I.; Rindi, G.; Bordin, C., *Genetic alterations in combined neuroendocrine neoplasms of the lung. Modern Pathology* 2008, 21, 414-422.
- Reck, M.; Heigener, D.F.; Mok, T.; Soria, J.C.; Rabe, K.F., *Management of non-small-cell lung cancer: recent developments. Lancet* 2013, 382, 709-719.
- Haghighi, S.M.; Allameh, A.; Mortaz, E.; Garssen, J.; Folkerts, G.; Barnes, P.J.; Adcock, I.M., *Pharmacogenomics and targeted therapy of cancer: Focusing on non-small cell lung cancer. European Journal of Pharmacology* 2015, 754, 88-97.
- Kumarakulasinghe, N.B.; van Zanwijk, N.; Soo, R.A., *Molecular targeted therapy in the treatment of advanced stage non-small cell lung cancer (NSCLC). Respirology* 2015, 20, 370-378.
- Oxnard, G.R.; Binder, A.; Janne, P.A., *New Targetable Oncogenes in Non-Small-Cell Lung Cancer. Journal of Clinical Oncology* 2013, 31, 1097-1104.
- Byers, L.A.; Rudin, C.M., *Small cell lung cancer: Where do we go from here? Cancer* 2015, 121, 664-672.
- Zarogoulidis, P.; Chatzaki, E.; Hohenforst-Schmidt, W.; Goldberg, E.P.; Galaktidou, G.; Kontakiotis, T.; Karamanos, N.; Zarogoulidis, K., *Management of malignant pleural effusion by suicide gene therapy in advanced stage lung cancer: a case series and literature review. Cancer Gene Therapy* 2012, 19, 593-600.
- Christensen, C.L.; Zandi, R.; Gjetting, T.; Cramer, F.; Poulsen, H.S., *Specifically targeted gene therapy for small-cell lung cancer. Expert Review of Anticancer Therapy* 2009, 9, 437-452.
- Tolosa, E.M.; Morse, M.A.; Lyster, H.K., *Gene therapy for lung cancer. Journal of Cellular Biochemistry* 2006, 99, 1-23.
- Woll, P.J.; Hart, I.R., *Gene-therapy for lung-cancer. Annals of Oncology* 1995, 6, 73-77.
- Lee, H.Y.; Sub, Y.A.; Lee, J.I.; Hassan, K.A.; Mao, L.; Force, T.; Gilbert, B.E.; Jacks, T.; Kurie, J.A., *Inhibition of oncogenic K-ras signaling by aerosolized gene delivery in a mouse model of human lung cancer. Clinical Cancer Research* 2002, 8, 2970-2975.
- Brockstedt, D.G.; Podsakoff, G.M.; Fong, L.; Kurtzman, G.; Mueller-Ruchholtz, W.; Engleman, E.G., *Induction of immunity to antigens expressed by recombinant adeno-associated virus depends on the route of administration. Clinical Immunology* 1999, 92, 67-75.
- Wang, L.L.; Figueredo, J.; Calcedo, R.; Lin, J.P.; Wilson, J.M., *Cross-presentation of adeno-associated virus serotype 2 capsids activates cytotoxic T cells but does not render hepatocytes effective cytolytic targets. Human Gene Therapy* 2007, 18, 185-194.
- Zhang, C.; Cortez, N.G.; Berns, K.I., *Characterization of a bipartite recombinant adeno-associated viral vector for site integration. Human Gene Therapy* 2007, 18, 787-797.
- Roth, J.A.; Swisher, S.G.; Merritt, J.A.; Lawrence, D.D.; Kemp, B.L.; Carrasco, C.H.; El-Naggar, A.K.; Fossella, F.V.; Glisson, B.S.; Hong, W.K., et al., *Gene therapy for non-small cell lung cancer: A preliminary report of a phase I trial of adenoviral p53 gene replacement. Seminars in Oncology* 1998, 25, 33-37.
- Swisher, S.G.; Roth, J.A.; Nemunaitis, J.; Lawrence, D.D.; Kemp, B.L.; Carrasco, C.H.; Connors, D.G.; El-Naggar, A.K.; Fossella, F.; Glisson, B.S., et al., *Adenovirus-mediated p53 gene transfer in advanced non-small-cell lung cancer. Journal of the National Cancer Institute* 1999, 91, 763-771.
- Akinc, A.; Zumbuehl, A.; Goldberg, M.; Leshchiner, E.S.; Busini, V.; Hossain, N.; Bacallado, S.A.; Nguyen, D.N.; Fuller, J.; Alvarez, R., et al., *A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. Nature Biotechnology* 2008, 26, 561-569.
- van der Aa, L.J.; Vader, P.; Storm, G.; Schifflers, R.M.; Engbersen, J.F.J., *Optimization of poly(amido amine)s as vectors for siRNA delivery. Journal of Controlled Release* 2011, 150, 177-186.
- Kakizawa, Y.; Furukawa, S.; Ishii, A.; Kataoka, K., *Organic-inorganic hybrid-nanocarrier of siRNA constructing through the self-assembly of calcium*

- phosphate and PEG-based block anioner. *Journal of Controlled Release* 2006, 111, 368-370.
23. Ji, L.; Roth, J.A., Tumor suppressor FUS1 signaling pathway. *Journal of Thoracic Oncology* 2008, 3, 327-330.
 24. Berg, R.W.; Ferguson, P.J.; Vincent, M.D.; Koropatnick, D.J., A „combination oligonucleotide“ antisense strategy to downregulate thymidylate synthase and decrease tumor cell growth and drug resistance. *Cancer Gene Therapy* 2003, 10, 278-286.
 25. Bennett, C.F.; Swayze, E.E., RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform. *Annual Review of Pharmacology and Toxicology* 2010, 50, 259-293.
 26. Heasman, J., Morpholino oligos: Making sense of antisense? *Developmental Biology* 2002, 243, 209-214.
 27. Johnston, W.K.; Unrau, P.J.; Lawrence, M.S.; Glasner, M.E.; Bartel, D.P., RNA-catalyzed RNA polymerization: Accurate and general RNA-templated primer extension. *Science* 2001, 292, 1319-1325.
 28. Owczarzy, R.; You, Y.; Groth, C.L.; Tataurov, A.V., Stability and Mismatch Discrimination of Locked Nucleic Acid-DNA Duplexes. *Biochemistry* 2011, 50, 9352-9367.
 29. Davies, A.M.; Gandara, D.R.; Lara, P.N., Jr.; Mack, P.C.; Lau, D.H.M.; Gumerlock, P.H., Antisense oligonucleotides in the treatment of non-small-cell lung cancer. *Clinical lung cancer* 2003, 4 Suppl 2, S68-73.
 30. Saini, S.S.; Klein, M.A., Targeting Cyclin D1 in Non-small Cell Lung Cancer and Mesothelioma Cells by Antisense Oligonucleotides. *Anticancer Research* 2011, 31, 3683-3690.
 31. Windhovel, U.; Haasper, C.; Duchrow, M.; Bruch, H.P.; Broll, R., Effect of cyclooxygenase-2 antisense oligodeoxyribonucleotides in a-549 lung cancer cells. *Anticancer Research* 2004, 24, 3789-3794.
 32. Cong, L.; Ran, F.A.; Cox, D.; Lin, S.L.; Barretto, R.; Habib, N.; Hsu, P.D.; Wu, X.B.; Jiang, W.Y.; Marraffini, L.A., et al., Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science* 2013, 339, 819-823.
 33. Platt, R.J.; Chen, S.D.; Zhou, Y.; Yim, M.J.; Swiech, L.; Kempton, H.R.; Dahlman, J.E.; Parnas, O.; Eisenhaure, T.M.; Jovanovic, M., et al., CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. *Cell* 2014, 159, 440-455.



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