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Respiratory viral diseases: access to RNA interference therapy

Vira Bitko, Sailen Barik*

Department of Biochemistry and Molecular Biology, University of South Alabama, College of Medicine, MSB 2370, 307 University Boulevard, Mobile, AL 36688-0002, USA

This review summarizes recent experimental achievements in the area of the development of new RNA interference (RNAi) therapeutics for the treatment of viral respiratory diseases. Delivery of short interfering RNA (siRNA) to their intended target tissue remains the biggest problem for most therapeutic applications of these compounds. Appropriate formulations and chemical modifications for improved stability will boost the probability of utilization of RNAi drugs in the clinical applications.

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Introduction

Viral respiratory infections strike millions of people each year and collectively cause more deaths than any other form of infectious disease. Common respiratory diseases are caused by a variety of viruses that have similar traits and affect the upper and lower respiratory tracts. Some common viruses are the influenza viruses, respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), respiratory adenoviruses or coronavirus. Effective vaccines against respiratory viruses are not available, because all of them are highly mutable. Treatment with ribavirin and amantadine is not very useful. The discovery of RNA interference (RNAi) has opened up new possibility for drug development. The ability of RNAi to silence the expression of specific rogue genes provides a novel approach to treat a wide range of human disorders. In this review, the authors draw attention to the recent developments in RNAi-based therapeutics for the treatment of various viral respiratory diseases.

*Corresponding author: S. Barik (sbarik@jaguar1.usouthal.edu)

RNA interference

RNAi is a mechanism for the inhibition of expression of specific genes by double-stranded RNAs (dsRNAs) [1]. In RNAi, long dsRNA is enzymatically cleaved by DICER (a dsRNA-specific RNase) into 20–22 nucleotide short interfering RNA (siRNA). The siRNA integrates into an endoribonuclease-containing protein complex called the RNA-induced silencing complex (RISC) whereby the strands are separated and the antisense strand guides RISC to the complementary target mRNA sequence. A key component of RISC, called Argonaute, which is also an RNase, then slices the target mRNA, leading to the suppression of expression of the corresponding genes and subsequent decrease in the corresponding protein level. Thus, RNAi offers generally the advantage of high specificity and enhanced potency as prophylactic and therapeutic treatment in targeting previously obstinate disease-causing genes.

Targeting respiratory viruses in tissue culture

The effectiveness of siRNAs as antiviral drugs was first evaluated in tissue culture. Results of investigations by several research groups demonstrated great potential of siRNA in combating such serious human and animal respiratory viruses as: RSV, severe acute respiratory syndrome (SARS) coronavirus, influenza, adenovirus, avian metapneumovirus and porcine respiratory virus, some of which are summarized here.

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Respiratory syncytial virus (RSV)

RSV is a nonsegmented negative-stranded RNA virus and a member of Paramyxoviridae family [2,3]. It is the most common cause of bronchiolitis and pneumonia among children one-year old and younger. By age 2, most children will have serologic evidence of RSV infection. Older adults and individuals with a compromised immune system or with heart or lung problems have an increased risk of developing complications from RSV infection [4]. RSV causes repeated re-infection throughout life, with symptoms ranging from moderate to severe croup, bronchiolitis, pneumonia and asthma. Although the development of anti-RSV vaccine is a high priority, none is currently available [5,6].

The first report of successful RNAi application as antiviral agent against RSV in tissue culture was published in 2001 [7]. siRNAs directed against viral fusion (F) and phosphoprotein (P) were shown to be highly specific, and the ablation is highly efficient. These siRNA were not only able to inhibit but also prevent RSV infection.

Influenza virus

Another respiratory virus with segmented negative-stranded RNA genome is influenza (flu) virus, a member of the Orthomyxoviridae family and responsible for 36,000 deaths annually in US. According to CDC, 5–20% of US population gets the flu and more than 200,000 are hospitalized from flu complications every year [8]. Pandemics of flu have been recognized since the earliest recorded history and because of the mutability of the virus still represent an alarming threat [9]. When the antigenic match between vaccine and circulating viruses is close, vaccines against influenza virus can prevent infection in 70–90% of healthy persons younger than 65 years age, compared with only 30–40% of older persons [10]. Furthermore, as the viral antigens undergo mutations frequently, vaccines against influenza need to be reformulated every year given that the previous year's vaccine becomes ineffective against any new virus subtype. In cell culture and in embryonated chicken eggs, siRNA specific to nucleocapsid (NP) and a component of the viral RNA-dependent RNA polymerase (RdRP) (PA) abolished the accumulation of not only the corresponding mRNA but also virion RNA and its complementary RNA. These siRNAs also broadly inhibited the accumulation of other viral, but not cellular, RNAs [11,12].

Two other groups of researchers used a different approach to inhibit influenza virus infection in tissue culture. They targeted the cellular protein, Ran-binding protein 5, involved in the nuclear import and assembly of the viral RdRP, essential for the viral life cycle [13]. siRNA-mediated knockdown of Ran-binding protein 5 indeed resulted in the delayed accumulation of viral RNAs in infected cells, which confirms that not only viral but also cellular genes may be targeted for therapeutic intervention in viral diseases.

Severe acute respiratory syndrome (SARS) coronavirus

SARS is a viral respiratory disease first reported in November 2002 in China. It is the first severe new disease to emerge in the 21st century. The first outbreak of the disease in Asia resulted in over 8000 infections, about 10% of which resulted in death; however, no case in the US has been reported yet. The disease has no vaccine and no treatment. As in the case of other respiratory viruses, the SARS coronavirus (SARS-CoV) has an RNA genome. The virus belongs to the family Nidovirales of positive-stranded RNA viruses known for their frequent mutations, creating difficulties for vaccine development [14]. Thus, the need for an effective anti-SARS drug cannot be overstated. To explore the possibility of interrupting SARS-CoV replication by RNAi, specific siRNAs targeting the viral spike [15], NP [16], RdRP [17–19] and envelop [19] protein genes as well as the leader sequence [20] were synthesized and introduced into mammalian cells. It was found that all anti-SARS siRNA inhibited viral gene expression. Such results may suggest a new, RNAi-mediated approach for the treatment and prevention of SARS.

Human coronavirus NL63 (HCoV-NL63)

HCoV-NL63 is a recently discovered [21,22] fourth member of the Coronaviridae family that infects humans and is associated with acute respiratory illness in young children and the elderly and in immunocompromised individuals. HCoV-NL63 is present in a significant number of respiratory tract illnesses of unknown etiology. The virus uses the same cellular receptor as its close relative, the SARS-CoV. siRNAs specific for HCoV-NL63 S glycoprotein, which initiates entry of the virus into susceptible cells, showed a profound inhibition of viral replication in virus-infected cells [23].

Adenoviruses

The adenovirus family of medium-sized, nonenveloped double-stranded DNA viruses contains at least 49 immunologically distinct types that can cause human infections. Most human adenoviruses cause widespread respiratory tract infections. Although most infections are mild and require only symptomatic treatment, in some cases, such as adenovirus serotype 11 infection in immunocompromised patients, adenovirus infections can result in severe complications. There is no virus-specific therapy. Vaccines were developed for adenovirus serotypes 4 and 7, but were available only for preventing acute respiratory disease among military recruits. In cell culture, siRNA-mediated silencing of adenoviral E1A mRNA inhibited viral replication, which suggests that RNAi-mediated targeting of adenovirus E1A may have a potentially therapeutic effect in controlling adenovirus infections [24].

Targeting respiratory viruses in animal models

During the past several years, various studies have been published demonstrating efficacious silencing of disease genes by local or systemic administration of siRNAs or shRNAs in animal models of human disease, including respiratory viral infections that are summarized here.

Systemic delivery of siRNA

The first evidence of successful systemic application of siRNA against respiratory viral disease was demonstrated for influenza A infection. siRNAs and DNA encoding shRNA, directed against conserved influenza sequences, were delivered to the lung by intravenous low-pressure injection and was able to prevent or treat an influenza virus infection when given, respectively, before or after infection [25]. These findings demonstrate that systemic delivery of siRNA and shRNA might be useful in the future for the development of antiviral therapeutics, although polyethyleneimine (PEI), the delivery reagent used in this study has been shown to have undesirable toxicity in some other studies. In addition, in four different studies combined systemic and local (intranasal) delivery of siRNA were used to combat influenza infection in murine model [11,25–27]. siRNA directed against viral nucleoprotein, acidic polymerase and matrix protein were able to protect mice from highly pathogenic strains of influenza A virus.

Local delivery of siRNA

Local delivery of siRNA represents a more attractive mode of application because it is less invasive than systemic intravenous delivery and possibility of adverse systemic responses is also minimized or eliminated. It has been shown that epithelial cells of respiratory tract might be transduced *in vivo* by siRNAs to significant extents even without transfection reagents [28,29]. This suggests that the lung may have a means for siRNA uptake, not generally present in most mammalian cells. The first therapeutic action of siRNA in the lung was demonstrated for SARS-CoV [28]. siRNA against viral spike and NP protein with high potency in tissue culture experiments were used in an animal model of infection. Intranasal delivery of siRNA into virus-infected rhesus macaque SARS model [30], that emulates the sequence of pathogenesis in humans with SARS, showed the abrogation of viral infection in upper airway epithelial cells [28].

In a leading study, RNAi was successfully used against two other respiratory viruses: RSV and PIV. In the BALB/c mouse model of RSV and PIV infections nasally delivered siRNAs directed against essential viral genes radically reduced viral titers [29]. siRNA also strongly reduced the clinical signs of infection. Weight loss and increased respiratory rate, which are pathological features of RSV and PIV infections in mouse, were dramatically improved, as were the allergic indicators such as leukotriene levels in bronchoalveolar lavage fluid.

Mice treated intranasally with siRNA against another RSV protein, the nonstructural protein 1 (NS1) before or after infection with RSV showed substantially decreased virus titers in the lung and decreased inflammation and airway reactivity compared to controls [31]. To investigate the persistence of siRNA prophylaxis, the authors treated mice with siRNA before RSV infection and demonstrated that prophylactic effect of siRNA against RSV NS1 protein can last for at least four days, although treatment at day −7 still lowered viral titer by tenfold, compared to the control.

Although researchers widely use siRNA technology in their basic studies to understand gene function, progress in the development of RNAi-based therapeutics has been slow. Alnylam Pharmaceuticals translated the successful animal results described above [29] into human trials to evaluate the antiviral activity of ALN-RSV01, an siRNA therapeutic for the treatment of RSV infection. Results with RSV-infected adult volunteers were claimed to be promising. The company has begun a Phase II study evaluating the safety and efficacy of ALN-RSV01 in experimentally infected adults in the second quarter of 2007 [32] and the results are slated to be available in the early first quarter of 2008.

Alnylam Pharmaceuticals is also working with Novartis AG on ALN-FLU01, an RNAi drug against influenza [33], likely to be formulated similarly as ALN-RSV01. Another preclinical RNAi player in flu is Nastech Pharmaceutical that announced positive results in a nonclinical study of siRNA-based treatment of influenza infection [34].

Conclusions

Modulation of gene expression has become the new promise in the development of therapeutics against many serious diseases. To take the full advantage of this approach, however, the siRNAs must be appropriately and efficiently delivered, which continues to be a significant hurdle. The use of a novel chitosan/siRNA nanoparticle nasal drug delivery system has been reported [35]. Chitosan is a sugar-based polymer, able to form selfassembling nanoparticles that entrap siRNA molecules. These nanoparticles can be taken up rapidly by cells. Development of InfaSurf, the pulmonary surface-active material that acts selectively and specifically in the lung allowed efficient siRNA transfection into mouse alveoli [36]. These novel approaches need further testing.

Another challenge in the *in vivo* application of siRNA is the need to increase their stability. siRNAs can be chemically modified by changes of the oligo backbone, replacement of individual nucleotides with nucleotide analogs and others. Such modifications may enhance mRNA targeting efficiency of siRNA.

Off-target effect is the other obstacle that must be overcome or minimized in developing siRNA therapies. This is due to the fact siRNAs are known to target mRNAs with imperfect complementarity, which may lead to uninten-

tional knockdown of vital cellular functions. Clearly, long-term and detailed safety evaluations will be required before the RNAi technology can be transferred to the clinic.

Outstanding issues

- Will RNAi prove to be better than the classical DNA antisense? A true answer to this question will require a head-to-head comparison of their silencing ability, immune reactions, efficacy in animals and side effects.
- What would it take to draw more attention to chemically modified siRNAs that have improved stability and pharmacology?
- Will siRNAs have synergistic or antagonistic effects if administered with other pharmaceuticals?
- Will siRNAs compete with endogenous miRNA pathway(s) and cause imbalance of the normal physiology in specific tissues?

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