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Review Article

Gene therapy and respiratory neuroplasticity

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ABSTRACT

Breathing is a life-sustaining behavior that in mammals is accomplished by activation of dedicated muscles responsible for inspiratory and expiratory forces acting on the lung and chest wall. Motor control is exerted by specialized pools of motoneurons in the medulla and spinal cord innervated by projections from multiple centers primarily in the brainstem that act in concert to generate both the rhythm and pattern of ventilation. Perturbations that prevent the accomplishment of the full range of motor behaviors by respiratory muscles commonly result in significant morbidity and increased mortality. Recent developments in gene therapy and novel targeting strategies have contributed to deeper understanding of the organization of respiratory motor systems. Gene therapy has received widespread attention and substantial progress has been made in recent years with the advent of improved tools for vector design. Genes can be delivered via a variety of plasmids, synthetic or viral vectors and cell therapies. In recent years, adeno-associated viruses (AAV) have become one of the most commonly used vector systems, primarily because of the extensive characterization conducted to date and the versatility in targeting strategies. Recent studies highlight the power of using AAV to selectively and effectively transduce respiratory motoneurons and muscle fibers with promising therapeutic effects. This brief review summarizes current evidence for the use of gene therapy in respiratory disorders with a primary focus on interventions that address motor control and neuroplasticity, including regeneration, in the respiratory system.

Contents

1. Introduction

Breathing is a life-sustaining behavior that in mammals is accomplished by activation of muscles responsible for inspiratory and expiratory forces acting on the lung and chest wall. Motor control is exerted by specialized pools of motoneurons in the medulla and spinal cord innervated by projections from multiple centers primarily in the brainstem that act in concert to generate both the rhythm and pattern of ventilation. Motor units innervating respiratory muscles are thus responsible for executing the motor behaviors responsible for ventilation (i.e., to maintain appropriate blood gas levels across a range of demands from rest to exercise, sleep and in varying environments). Respiratory motor units also contribute to many other tasks including coughing, sneezing, vomiting, defecation and parturition (Butler et al., 2001; Mantilla et al., 2010, 2014b; Mantilla and Sieck, 2011; Milano et al., 1992; Sieck and Fournier, 1989; Sieck, 1994). These tasks generally require much higher levels of force generation and are indispensable to maintain airway patency. Perturbations that prevent the accomplishment of the full range of motor behaviors by respiratory muscles commonly result in significant morbidity and increased mortality (Brown et al., 2006; Hamel et al., 2005; Linn et al., 2000; Ray et al., 2006; Winslow and Rozovsky, 2003). In this regard, both neuroplasticity and

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regeneration act within a balance of stabilizing (homeostatic) influences and adaptive responses working in concert to maintain appropriate levels of function. Respiratory failure is a common terminal feature of many neurological and neuromuscular conditions, highlighting the importance of in-depth knowledge of the mechanisms responsible for maintaining respiration and of appropriate and timely intervention. In patients with neurodegenerative disorders such as amyotrophic lateral sclerosis and stroke, death is most frequently the result of respiratory failure (Gregory, 2007). Following spinal cord injury, for instance, the main determinant of a patient's long-term survival and morbidity is the need for mechanical ventilation (Claxton et al., 1998; DeVivo and Ivie, 1995), and pulmonary function is commonly impaired even in cases of incomplete injury and paraplegia (Linn et al., 2000). However, some recovery of respiratory muscle function is possible (Oo et al., 1999), suggesting substantial, even delayed, regenerative neuromuscular capacity. This brief review will summarize current evidence for the use of gene therapy in respiratory disorders with a primary focus on interventions that address neuroplasticity including regeneration in the respiratory system.

Gene therapy has received widespread attention in recent years and substantial progress has been made with the advent of improved tools for vector design. Excellent recent reviews address the state-of-the-art of gene therapy applications (c.f., Franz et al., 2012; Samulski and Muzyczka, 2014). In order to achieve reliable regulation of gene expression, a variety of viral vectors have been used in many animal models and clinical applications are increasing. Gene therapy comprises delivery of exogenous nucleic acids or gene products using various means, including viruses and cell transplantation, and has recently shown great promise in addressing genetic disorders. With gene therapy it may be possible to replace defective genes with functioning ones, eliminate malfunctioning genes or introduce new genes to change cell expression and induce plasticity that may then help restore function via alternate mechanisms. Recent developments in gene therapy and novel targeting strategies have contributed to deeper understanding of the organization of respiratory motor systems, provided important therapeutic opportunities across various genetic disorders, and, by using novel targeting strategies, contributed to understanding the organization of respiratory motor systems.

2. Targeting strategies for gene therapy

Genes can be delivered via a variety of plasmids (small circular DNA carrying recombinant genes of interest), synthetic or viral vectors and cell therapies. Gene delivery vectors include synthetic lipid or polymer coatings to protect DNA from degradation from ubiquitous DNAses, as well as naturally-occurring and engineered viruses that can be designed to target specific cells. Indeed, the selection of the vector is paramount in determining the localization, expression levels and timing (both onset and duration) characteristics for the gene of interest. Various adenoviruses, adeno-associated viruses (AAV), herpes virus and lentivirus, in particular, have been the subject of widespread investigation. In general terms, adenovirus and herpes virus are not conducive to stable transgene expression in non-dividing cells (e.g., neurons) allowing only transient expression and they commonly induce substantial immune responses. Insertional mutagenesis is a risk with the use of lentiviruses. In recent years, AAV have become one of the most commonly used vector systems, primarily because of the extensive characterization conducted to date and the versatility in targeting strategies.

Infection and replication by AAV require a helper virus (e.g., an adenovirus), consistent with other members of the Dependovirus genus (Wu et al., 2006). In the absence of a helper virus, AAV may persist by integrating into the cell genome (usually site-specific integration) or remain as episomal DNA. Many AAV serotypes and variants have been characterized recently (Gao et al., 2004; Schmidt et al., 2006). By definition, a new serotype represents a virus variant that after isolation does not exhibit cross-reactivity with neutralizing sera for all other serotypes currently characterized. AAV serotypes (AAV1-11) are numbered based on their isolation, but many AAV variants exist that have not been submitted to rigorous serological characterization. Of note, the transduction efficiency of different AAV serotypes is only partially related to the existence of neutralizing antibodies to related serotypes, and species and route of administration play important roles in determining efficiency (Halbert et al., 2000).

Numerous AAV serotypes, both naturally occurring and engineered, are now available (Samulski and Muzyczka, 2014), and display enormous variability in cell specificity mostly dictated by capsid structure and binding to cellular receptors. Ultrastructural analyses of the AAV capsid have now achieved detailed resolution (~1 nm) (Shevtsova et al., 2005). In fact, significant progress has also been made in understanding the characteristics that determine viral tropism. Cellular entry is initiated by interactions between the capsid and the glycosaminoglycans on the cell surface. For instance, AAV serotypes 2, 3, 8 interact with a 37-kDa/67-kDa laminin receptor, AAV3 also interacts with heparan sulfate proteoglycans, AAV4 with O-linked sialic acid and AAV5 platelet-derived growth factor receptor (Daya and Berns, 2008). Intracellular trafficking depends on the interaction with co-receptors and association with endo-lysosomal or retrograde pathways. Accordingly, serotype selection is an important factor in conferring cell specificity and transduction efficiency across tissues.

There is great interest in identifying viral properties that can be used to improve target selectivity. Neuronal tropism depends on delivery route (e.g., intrathecal vs. systemic) and, at least in part, on the ability to cross the blood brain barrier of the different serotypes. Both anterograde and retrograde axonal transport are important characteristics of AAV (Castle et al., 2016). For instance, previous studies report that AAV serotypes 1, 5, 6, 7 and 9 show retrograde transport to motoneurons (Boulis et al., 2003; ElMallah et al., 2012; Fortun et al., 2009; Gransee et al., 2013; Lu et al., 2003; Towne et al., 2010). However, efficacy of motoneuron transduction is limited; several approaches to improve efficiency have been developed including self-complementary viruses (Hollis et al., 2008) and capsid engineering to include the small peptide Tet1, which binds to gangliosides present on motoneuron membranes (Davis et al., 2015). It is worth noting that improved motoneuron transduction with double-stranded self-complementary AAV (up to 20-fold) likely does not reflect changes in retrograde transport (Hollis et al., 2008), but increased DNA delivery to the cell. This improvement comes at the cost of ~50% reduction in the packaging capacity and thus the size of the transgene encoded within each AAV particle (to ~2.5 kb).

Cellular specificity may also depend on the approach used to deliver viral vectors, since most particles will usually remain at the site of injection. Indeed, localization of vector delivery can be achieved by appropriate selection of injection route (e.g., intravenous vs. intraspinal) and, in particular, novel delivery routes that harness specific properties of the vector system such as retrograde transport in respiratory motoneurons are actively investigated. Several studies using intramuscular and intrapleural injection show that various vectors achieve selective transduction of motoneurons innervating respiratory muscles (ElMallah et al., 2012; Gransee et al., 2013; Martinez-Galvez et al., 2016). The underlying mechanisms for motoneuron transduction using AAV still remain to be elucidated and whether uptake is restricted to axon terminals (e.g., following intramuscular delivery) or the axon itself (e.g., following intrapleural or systemic injection) is not presently known.

In a recent study (Gransee et al., 2013), we conducted a survey of multiple AAV serotypes delivered by intrapleural injection and found that AAV7 when injected intrapleurally targets phrenic motoneurons. Green fluorescent protein (GFP) expression was evident only in retrogradely-labeled phrenic motoneurons in the cervical spinal cord. Evidence of GFP immunoreactivity was present in the cell bodies and dendrites of phrenic motoneurons, and in aggregate, at least 11% of motoneurons showed evidence of GFP expression (Gransee et al., 2013). In a separate study also using AAV7, GFP fluorescence was evident in the