Astrocytes are now considered as key players in brain information processing because of their newly discovered roles in synapse formation and plasticity, energy metabolism and blood flow regulation. However, our understanding of astrocyte function is still fragmented compared to other brain cell types. A better appreciation of the biology of astrocytes requires the development of tools to generate animal models in which astrocyte-specific proteins and pathways can be manipulated. In addition, it is becoming increasingly evident that astrocytes are also important players in many neurological disorders. Targeted modulation of protein expression in astrocytes would be critical for the development of new therapeutic strategies. Gene transfer is valuable to target a subpopulation of cells and explore their function in experimental models. In particular, viral-mediated gene transfer provides a rapid, highly flexible and cost-effective, in vivo paradigm to study the impact of genes of interest during central nervous system development or in adult animals. We will review the different strategies that led to the recent development of efficient viral vectors that can be successfully used to selectively transduce astrocytes in the mammalian brain.

Keywords: viral vectors, astrocytes, CNS, tropism, gene therapy

Astrocytes make up most of the cells in the brain. In addition, they have well-characterized roles for astrocytes in regulating brain metabolism and blood flow, there is now an increasing body of evidence that astrocytes are dynamic regulators of synaptogenesis, synaptic function and network activity. This is conceptualized in the tripartite synapse model, where pre-synaptic and post-synaptic elements of neurons are surrounded and regulated by astrocyte processes (Araque et al., 1999; Barres, 2008).

Astrogenesis occurs relatively late in development after most neurons have completed (Freeman, 2010). Defects in astrocyte maturation, tripartite synapse formation and plasticity during early postnatal development may be responsible for some psychiatric and neurodegenerative diseases. There is a growing body of evidence to support the view that a loss of normal astrocyte functions or a gain of abnormal effects can contribute to disease processes, and there are now numerous examples of astrocyte contributions to pathological mechanisms in amyotrophic lateral sclerosis (ALS), Huntington’s disease (HD), and brain tumors to cite few of them (for review see Sofroniew and Vinters, 2010).

Despite progress and potential significance, cellular, developmental, and systems-level studies of astrocytes still lag far behind those of neurons. New sophisticated genetic tools to label and manipulate astrocytes in vivo were recently developed. Additional tools that allow for temporally controlled deletion of genes, specifically in rodent astrocytes, along with improved high resolution imaging techniques, are enabling researchers to address fundamental questions in astrocyte biology for the first time. However, these tools need to be more fully expanded and exploited to better understand astrocyte biology in vivo. The situation is complicated by the recent findings that astrocytes do not represent a homogeneous cell population across brain regions as well as within the same brain region (Zhang and Barres, 2010). So, despite evidence showing pronounced region- and layer-specific morphological heterogeneity as well as region-specific actions of astrocytes on neuronal functions, currently available tools have had limited utility for examining functional diversity among astrocytes.

To understand the role of astrocyte signaling in brain function, it is critical to study astrocytes in situ where their complex morphology and intimate association with neurons remains intact. Understanding neuron–glia interactions in vivo requires dedicated experimental approaches to manipulate each cell type independently. These approaches include targeted transgenesis and viral
transduction to overexpress or block the expression of a specific gene in astrocytes. The past and current approaches of targeted transgenesis were recently reviewed in a comprehensive paper (Pfister and Sleazak, 2012) and will not be detailed here.

Yet, a very important application of transgene expression is the visualization of a large population of astrocytes in vivo by a fluorescent protein. The use of viral artificial chromosomal vectors (BACs) for the production of transgenic mice has opened new opportunities to study gene expression and functions in the brain. The resulting gene expression control central nervous system (CNS) atlas program GENSAT represents a powerful resource for the scientific community (http://www.gensat.org). However, it remains difficult and time-consuming to target specific cell subpopulations through transgenesis, and differences in recombination efficiency between transgenic lines complicate the analysis. We will therefore rather focus on an alternative approach to genetically manipulate astrocytes that relies on the use of viral vectors. Indeed, the development of highly efficient viral vectors for gene transfer in the CNS is providing new systems for localized and controlled gene expression. Even if such approach requires the stereotactic injection of the viral vectors in each animal, it significantly reduces the costs of in vivo experiments, and it can be used in combination with mouse models for conditional gene targeting, providing high flexibility and reversibility to replace, modify, induce, or block expression of target genes. We will therefore review the recent development in this field that led to the emergence of effective and selective viral vectors for transducing astrocytes in vivo.

VIRAL VECTORS: POTENT SYSTEM FOR IN VIVO GENE DELIVERY IN BRAIN

Viral vectors offer the possibility to control expression of a transgene in adult or developing brain areas and can exploit the unique ability of viruses to deliver genetic material into mammalian cells. Viral vectors are derived from various viruses and are engineered to preserve the transduction efficiency while preventing cell toxicity, with a partial (first generation of HSV-1 vectors) or complete (amplicons) deletion of viral genes allowing the insertion of very large transgenes (around 150 kb). The HSV-1 amplicons are neither pathogenic nor toxic for the infected cells and are retrogradely transported to the host genome thus reducing the risk of insertional mutagenesis (Manservigi et al., 2010). However, HSV amplicons are difficult to produce, elicit low levels of adaptive immune responses and most of the human population is seropositive which limits their clinical applications for chronic disorders (Manservigi et al., 2010).

A few years after the apparition of HSV vectors, adeno-associated viral vectors (AAVs) were derived from the Ad type 5 serotype (Le Gal La Salle et al., 1993; Horellou et al., 1994). These vectors also have a high cloning capacity (approximately 30 kb of double-stranded DNA for guilless Ad) but the tropism of these vectors is not naturally oriented to the brain (Arnberg, 2012). Interestingly, a live (replication-competent Ad) vaccine has been safely administered to humans (Rubin and Rorke, 1994). This vaccine program reflects the strong immune response induced by Ad in humans (White et al., 2011), a reason why these vectors are promising candidates for tumor therapy, and are proposed for the treatment of glioblastoma (Caudilli et al., 2006; Knezevic et al., 2010).

In the mid 1990s, the first AAV (from serotype 2) and LVs were reported (Page et al., 1990; Kapli et al., 1994; Naldini et al., 1996). The AAV vectors are derived from the smallest non-enveloped viruses (approximately 20 nm) and have a cloning capacity of 5 kb of single-stranded DNA. The AAV2 naturally infects humans but is non-pathogenic. It is classified as a dependovirus because it requires a co-infection with a helper virus such as Ad or HSV to perform its infectious replication cycle. The AAV persists for years in transduced cells mostly as an extrachromosomal episome (Nakai et al., 2001; Schnepp et al., 2005). To date, more than 100 serotypes of AAV have been identified, each of them possessing a specific tropism in the CNS due to the binding of the capsid with specific receptors (Wu et al., 2006a,b). Fourteen clinical trials using AAV gene transfer were performed to assess their potential therapeutic value in various neurodegenerative diseases (Crystal et al., 2004; Tuszyński et al., 2005; Kapli et al., 2007). In 2012, the first AAV gene therapy product was marketed by the European Medicine Agency (EMA) for the treatment of patients suffering from lipoprotein lipase deficiency (Yla-Herttuala, 2012).

The first viral vector was obtained by exploiting the natural tropism of brain cells from the Herpes simplex virus type 1 (HSV-1; Geller and Breakefield, 1988; Fedoroff et al., 1992). The HSV-1 genome is complex and large, but replication-incompetent vectors, with a partial (first generation of HSV-1 vectors) or complete (amplicons) deletion of viral genes allow the insertion of very large transgenes (around 150 kb). The HSV-1 amplicons are neither pathogenic nor toxic for the infected cells and are retrogradely transported to the CNS from the peripheral nervous system (PNS; Frampton et al., 2005). These vectors have a widespread tropism for neurons (Jerusalinsky et al., 2012) and similarly to AAV and adenoassociated viral vectors, their genetic material does not integrate into the host genome thus reducing the risk of insertional mutagenesis (Manservigi et al., 2010). However, HSV amplicons are difficult to produce, elicit low levels of adaptive immune responses and most of the human population is seropositive which limits their clinical applications for chronic disorders (Manservigi et al., 2010).

Viral vectors (Figure 1). Among the most widely used vectors for CNS applications are the lentiviral (LVs) and adeno-associated viral vectors (AAVs) which have particularly attractive properties which include, the capacity to infect non-dividing cells, the absence of cytotoxic or immune response, long-term transgene expression and large diffusion in the brain. At least for LV, the cloning capacity is sufficient to integrate most of the genes of interest (Deglon and Hantraye, 2005). Viral vectors provide a gene transfer tool that is independent of age and species considered (Kay et al., 2001; Kirik et al., 2003; Lundberg et al., 2008). Along with somatic gene transfer in developing or adult animals, viral vectors can also be used for transgenesis in species in which classical methods are not suitable, in particular large animals (Yang et al., 2008; Wongsriao et al., 2011).

Natural viruses have a specific pattern of infection, which reflects the recognition and interaction between viral capsid/envelope and receptors expressed on susceptible cells. Similarly, the tropism of viral vectors is primarily determined by the interaction of the viral surface proteins with receptor molecules expressed on target cells but other mechanisms could be used for subpopulation-restricted gene transfer in the brain. In particular, cell-type-specific promoters, post-transcriptional regulatory elements, replacement of retroviral envelope proteins with heterologous viral surface proteins, a phenomenon called pseudotyping (Page et al., 1990) or the use of various serotypes (AAVs and Ad harboring different capsids) have been proposed to dissect and elucidate gene functions in astrocytes.

A few years after the apparition of HSV vectors, adeno-associated viral vectors (Ad) were derived from the Ad type 5 serotype (Le Gal La Salle et al., 1993; Horellou et al., 1994). These vectors also have a high cloning capacity (approximately 30 kb of double-stranded DNA for guilless Ad) but the tropism of these vectors is not naturally oriented to the brain (Arnberg, 2012). Interestingly, a live (replication-competent Ad) vaccine has been safely administered to humans (Rubin and Rorke, 1994). This vaccine program reflects the strong immune response induced by Ad in humans (White et al., 2011), a reason why these vectors are promising candidates for tumor therapy, and are proposed for the treatment of glioblastoma (Caudilli et al., 2006; Knezevic et al., 2010).

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FIGURE 1 | Strategies to target astrocytes. Three steps of viral cycle are used to modify the tropism of viral vectors: (1) the entry, (2) the transcriptional and (3) post-transcriptional regulations. After binding to their respective receptors, LV, AAV, and Ad enter into host cells via receptor-mediated endocytosis. Viral DNA (AAV and Ad) or RNA (LV) are uncoated in the cytoplasm. The viral DNA remains as extrachromosomal episomes in the nucleus while viral RNA is integrated into the host genome after reverse transcription. For non-replicative vectors, in most cases only the transgene is expressed. In the case of oncolytic viruses, viral genes encoding structural proteins are necessary for the encapsidation and production of replicative particles. PIC, pre-integration complex.

Finally, the most extensively characterized LVs are derived from HIV-1, which is a subclass of retroviruses. Retroviruses are lipid-enveloped particles comprising a homodimer of linear, positive-sense, single-stranded RNA genomes of 7–11 kb. Following entry into target cells, the RNA genome is retro-transcribed into linear double-stranded DNA and integrated into the cell chromatin (Dreilis et al., 2010). To decrease the risk of insertional mutagenesis, integration-deficient LVs (IDLVs) were designed (Wanisch and Yanez-Munoz, 2009). These IDLVs are based on the use of integrase mutations that specifically prevent proviral integration, a process that results in the generation of increased levels of circular vector episomes in transduced cells. LVs were tested clinically for the treatment of adrenoleukodystrophy (ALD) and Parkinson’s disease (PD). In the case of ALD, an ex vivo approach was used, with the transduction of hematopoietic CD34+ cells and re-infusion of corrected cells in the patients. An
immunological improvement occurred in the two treated children aged 9–12 months in combination with a blockage of the demyelinating lesions observed by magnetic resonance imaging (MRI), 12–16 months after gene therapy (Carrier et al., 2009, 2012). In a second study, a dopamine replacement strategy, with an LV that encodes the three enzymes responsible for the production of dopamine was tested in a phase I clinical trial. Increasing doses of LV were injected into the striatum of 15 patients with mid-stage PD. An improvement in motor function was observed at 6-months relative to pre-treatment assessment (Poll, 2008; Jarrahy et al., 2009 and see http://www.oxfordbiomedica.co.uk).

**STRATEGIES TO TARGET ASTROCYTES**

The understanding of astrocyte functions in normal and altered brain strongly relies on the availability of experimental systems to specifically target astrocytes in vivo. However, the first generation CNS viral vectors had a strong neurotropism in vivo (Naldini et al., 1996; Hermens and Verhaagen, 1997; Rabinowitz and Samulski, 1998). Indeed, the injection of AA V2 into adult rodent brains was associated with neuronal transgene expression when using ubiquitous promoters (Barlett et al., 1998; Mandel et al., 1996; Bjorklund et al., 2000). Similarly, stereotaxic injection into rat or mouse brain of LVs pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) or CMV (cytomegalovirus) or PKG (phosphoglycerate kinase 1) promoters, leads to the specific transduction of neurons with very limited transgene expression in other cell types (Naldini et al., 1996; Kordower et al., 1999; Déglon et al., 2001). Finally, the Ad5 displays a partial neurotropism with the transduction of other cell types, especially astrocytes (Smith et al., 1996; Bohn et al., 1999; Soudais et al., 2001; Rubio and Martin-Clemente, 2002; Wang et al., 2012).

However, it is important to mention that a number of parameters could alter the tropism. These include, amongst other factors, the purity of the vector, the mode of production, the site of administration, species, the developmental stage, and normal or pathophysiological conditions. Unfortunately, data gathered in primary cultures (neurons and astrocytes) are not predictive of the in vivo tropism and a systematic evaluation of each vector is still required. Indeed, VSV-G/LV-GFP under the control of various promoters efficiently transduces primary rat astrocytes and to a lesser extent mouse astrocytes (Englund et al., 2000; Li et al., 2010) while transgene expression is mainly restricted to neurons in vivo (Naldini et al., 1996; Kordower et al., 1999; Déglon et al., 2000). This phenomenon was also observed with AA V2, which efficiently targets astrocytes in vivo but not in vivo (Gong et al., 2004). The purification method has also a major impact on the tropism of AA V5. In the mouse hippocampus, the CCI-purified AA V5-CMV-GFP displayed an astroglial pattern in contrast to the expected neuronal expression obtained with an iodixanol purification method (Klein et al., 2008). Foust et al. (2009) found that injection of AA V9-CMV early enhancer/chicken β actin promoter (CAG)-GFP into the tail vein of adult mice mainly transduces astrocytes throughout the CNS (Foost et al., 2009), whereas the tropism is mainly neuronal after intracerebral injection or intravenous injection in neonatal mice (Klein et al., 2004). Finally, discrepancies have been observed on the transduction efficiency and tropism of various AAV serotypes between species (rodent, cat, and primates; Davidson et al., 2000; Vite et al., 2003; Burger et al., 2004; Gray et al., 2011). Additional studies are therefore still warranted to fully characterize the tropism of these vectors in the CNS. However, three strategies to direct viral vectors toward astrocytes have already been developed: shifting the tropism by favoring the entry of viruses in astrocytes, limiting transgene expression with astrocyte-specific promoters or blocking transgene expression in unwanted cells (Figure 2).

**ALTERING THE ENTRY OF VIRAL VECTORS**

The tropism of a virus is first determined by its binding with a specific receptor at the surface of the host cell (Lutzsch et al., 2011; Artinger, 2012). Knowledge of the structure and viral capsids or envelopes and their corresponding receptors provide essential information to specifically target individual cell types and/or diseased tissues. For example, the tropism of Ad5 vectors is regulated by the binding to its primary cellular receptor, the coxsackie and adenoviral vectors receptor (CAR). Tissues refractory to Ad5 infection do not express CAR. The limited expression of CAR in dopaminergic neurons of the substantia nigra of mice explains the poor transduction of these cells and transgene expression in astrocytes and other non-neuronal cells (Lewis et al., 2010). However, the expression of CAR in the nervous system and in particular in glial cells has not been extensively examined and CAR-independent forms of Ad have been developed to shift the tropism (Grellier et al., 2011).

As mentioned above, more than 100 serotypes of Ad and AA V were characterized but only a dozen of them infect cells of the CNS. Indeed, for most of them, only limited data are available concerning their receptors and their pattern of expression in the brain. The earliest and most used serotype is the AA V2, which has a natural tropism for neurons (Barlett et al., 1998; Kugler et al., 2003). The binding of AA V2 to its primary receptor, the heparan sulfate proteoglycan (HSPG) has been well-characterized, and is centered around two amino acids on the spikes of the AA V2 capsid (Kern et al., 2003; Opie et al., 2003). However, HSPG is necessary, but not wholly sufficient, for the transduction of permissive cells. In addition, fibroblast growth factor receptor 1 (FGFR1) was identified as a co-receptor of AA V2 (Qing et al., 1999). The tropism of AA V5 in vivo correlated with the pattern of expression of platelet-derived growth factor receptor (PDGFR)-alpha (Di Pasquale et al., 2003). The AA V1, 5, 7, 8, and 9 not only infect astrocytes in vivo but also neurons and other cells (Davidson et al., 2000; Wang et al., 2003; Shertova et al., 2005; Gareley and Wolfe, 2006; Gray et al., 2011). The AA V9 is unique compared to other AA serotypes in that it is capable of crossing the blood–brain barrier and transducing neurons and/ or astrocytes in the brain depending of the developmental stage (Foost et al., 2009). Recently, it has been shown that AA V9 uses galactose at the N-linked glycans as a receptor (Bell et al., 2011; Shen et al., 2011). The identification of the amino acids of the AA V9 capsid necessary for binding to galactose opens the possibility to modify the tropism (Bell et al., 2012). Finally, AA V4 and AA Vrh43 preferentially target astrocytes (Liu et al., 2005; Lawlor et al., 2009) but the receptors for these serotypes are unknown. AA V4 RSV-βGal and AA Vrh43-CAG-GFP exclusively transduce astrocytes when injected into the subventricular zone (SVZ) or the striatum.
However, AAVrh43-CAG-eGFP infects approximately 3 mm³ of the striatum and 2,000 astrocytes per mm³ while AAVrh8-CAG-eGFP infects 6 mm³ of the striatum and 150,000 neurons per mm³ (Lawlor et al., 2009).

Lentiviral vectors are increasingly being used in neuroscience research and are unique in the sense that they are enveloped viruses that can be pseudotyped (i.e., the original envelope protein can be replaced by heterologous glycoproteins). The most used pseudotype for LV is VSV-G which confers some interesting properties to the vector (Figure 2). It dramatically broadens LV tropism by facilitating transduction of various cell types in different species, it stabilizes the vector particles from shear forces during centrifugation thereby allowing vector concentration and it directs LV to an endocytic pathway, which reduces the requirements of viral accessory proteins for transduction (Cockrell and Kafri, 2007). Initial studies suggest that VSV-G/LV enters into cells using phosphatidylserine (PS), but there is no correlation between the cell surface PS levels and VSV infection or binding (Coil and Miller, 2004). In addition, competition for PS using antagonists does not block the binding of VSV on target cells. Currently, the receptors responsible for VSV-G/LV entry in cells are unknown.

In the CNS, VSV-G/LVs expressing transgenes under the control of ubiquitous promoters have mainly a neuronal tropism with a limited transgene expression in astrocytes (Naldini et al., 1996; Déglon et al., 2000; Watson et al., 2002). Among the other envelopes used to pseudotype LVs, lymphocytic choriomeningitis virus (LCMV) and Mokola virus (MOK) envelopes result in a partial transduction of astrocytes. In vivo, LCMV/LVs infects specifically astrocytes in the substantia nigra and in the striatum (Miletic et al., 2004; Cannon et al., 2011). Injection of MOK/LV into the striatum or the hippocampus leads to the infection of cells that are mainly astrocytes (Pertusa et al., 2008; Collin et al., 2009). Although no quantifications were done using LCMV/LV, 70% of cells transduced by MOK/LV are astrocytes, 20% are neurons and 10% are other cell types of the striatum. In addition, it is important to note that the titers and the transduction efficiency of these latter vectors are usually lower than VSV-G/LV.

In conclusion, specific serotypes or envelopes only partially improve the astrocytic targeting of viral vectors. However, engineering chimeric capsids or envelopes targeting astrocytes is difficult and time-consuming. In order to optimize viral vectors tropism, strategies aiming at restraining transgene expression with astrocytic promoters, or by blocking expression in unwanted cells, mainly in neurons, were developed.

TARGETING ASTROCYTES WITH TRANSCRIPTION REGULATORY ELEMENTS

Different astrocytic promoters have been used to restrict transgene expression into glial cells. However, the packaging size of each viral vector limits the type of promoters which can be inserted. Analysis of the transcriptional regulatory elements of the glial fibrillary acidic protein (GFAP) promoter reveals that 5′-flanking regions

|FIGURE 2| Mechanisms used to restrain the transgene expression of AAV and LV in astrocytes. (1) To modify the entry, various AAV serotypes or LV pseudotyping with heterologous VSV-G (green) and MOK-G (blue) envelopes were used. The tropism of LV is mainly neuronal (green cells) with the VSV-G envelope and a partial shift toward astrocytes (blue cells) is observed with the MOK-G envelope. AAV1, 2, 5, and 8 mainly transduce neurons (green), while AAV4, 9, rh43 display a partial astrocytic tropism. (2) To restrict transgene expression, astrocytic promoters were investigated (cells in the upper part). Transgene expression under the control of a PGK promoter (pPGK, green mRNA) leads to a preferential expression in neurons, whereas a gfa2 promoter (pgfa2, blue mRNA) results in an astrocytic expression. (3) To block the transgene expression in unwanted cells (lower part), miRNA target (miRT) sequences are integrated in the 3′-UTR of the vector (red signal on the green mRNA). The mir124a is exclusively expressed in neurons. As a consequence the mir124 target (miR124T) is only recognized in neurons and the transgene expression is blocked (mRNA degraded). mir124a, microRNA 124; mir124aT, mir-124 target sequence; Tg, transgene.
of the gene are sufficient to direct transgene expression in astrocytes (Brenner et al., 1994). Two fragments compatible with AAV and LV vectors were created: gfa2 of 2.2 kb and gfaABC1D of 600 bp (Brenner et al., 1994; Lee et al., 2008). The cloning of the gfa2 fragment into Ad5 and AAVir43 vectors restricts transgene expression in rat astrocytes (Do Thi et al., 2004; Lawlor et al., 2009; Mambo et al., 2010; Arregui et al., 2011). However, no quantification was performed to determine the number of transduced astrocytes. In the study by Lawlor et al. (2009), the gfa2 promoter was cloned into the AAVir vector. The Gfa2-AAV vector infects mainly astrocytes in the striatum but a low transgene expression was still observed in neurons. The authors indicated that AAVir-gfa2-eGFP has high transduction efficiency with a wide diffusion in the striatum while AAVir43-gfa2-eGFP transduces only a limited number of cells. It was shown recently that injection of high titer of AAVir-gfa2-eGFP into the striatum or the substantia nigra provides an astrocyte-specific expression with no residual expression into neurons or microglial cells. In addition, the expression was stable until 12 weeks post-injection. Stereological analysis of transgene expression reveals that a mean of 15,000 astrocytes per mm² of striatal tissue were transduced (Drinkut et al., 2012), corresponding to ~75% of the astrocytes present in the transduced area (Sarchenko et al., 2000).

Astrocytic promoters were also used in combination with LCmv and MOK pseudotyped LVs (Figure 2). The vector LCmv/LV-gfa2-Cre was injected into the SVZ of Rosa26 mice that express the sequence LoxP-stop-LoxP-LacZ (Stein et al., 2005). The expression of Cre in transduced cells removes the SYFP cassette in Rosa26 mice and as a consequence, LacZ staining was observed in astrocytes of the SVZ after LCmv/LV injection. However, no quantification was performed although some neurons expressed the transgene. To develop an expression system activated in pathological conditions, Jakobsson et al. (2004) took advantage of GFAP up-regulation in reactive astrocytes. Using toxin-induced lesion models (β-hydroxy-dopamine and ibotenic acid lesions), they showed that the transgene expression is eight-fold higher in reactive astrocytes: a finding which correlates with the activity of the endogenous GFAP gene (Jakobsson et al., 2004). Recently, other astrocytic promoters were used in LV vectors, such as the glutamate transporter promoter, EAA1 (Colin et al., 2009). In this study, striatal injection of MOKLV-EAA1-GFP leads to the expression of the transgene mainly in astrocytes (75% of the transduced cells).

In conclusion, astrocyte-specific promoters alone or in combination with an “astrocytic” capsids or envelopes, significantly shift the tropism of viral vectors toward astrocytes in vivo. However, the targeting is, in most cases, not complete and a residual transduction (10–40%) of non-astrocytic cells is observed. In addition, most studies rely on the use of the GFAP promoter. Large initiatives are underway to characterize the regulatory elements of the whole human genome (Gerstein et al., 2012; Whitfield et al., 2012) and new astrocyte-specific promoters were recently described. For example, the aldehyde dehydrogenase 1 family, member L1 (ALDH1L1) promoter is highly active in all mature astrocytes (Cahoy et al., 2008) while the GLAST promoter was used to express transgene in GFAP-positive but also GFAP-negative astrocytes (Liu et al., 2006; Regan et al., 2007; Buffo et al., 2008). Analysis of GLAST and GFT1-GFP mice has revealed an unexpected non-overlapping pattern between the two transporters and confirmed the differential activation of the promoters during embryogenesis and in adulthood. GLAST activity was low in the forebrain and high in the cerebellum, whereas GFT1 expression was higher in the cortex than in the cerebellum, consistent with the prominent role of GFT1 in glutamate uptake in the forebrain. Combining data from the ENCODE project and the gene expression cartography in human and mouse brain will provide additional and essential information to identify minimal fragments necessary for cell-type-specific transgene expression in viral vectors (Hosy-lycz et al., 2012). This strategy has already been developed by the Pleaide Project, which integrated information from genomic databases to construct synthetic MiniPromoters for viral vectors containing only the indispensable regulatory elements to achieve gene expression (Portales-Casamar et al., 2010).
addition, each miRNA has differential suppressive activity ranging from 5 up to >150-fold (Gentner et al., 2009). In this context, miR124 is a promising candidate because it is highly expressed in neurons (Lagos-Quintana et al., 2002; Smirnova et al., 2005; Deo et al., 2006). The insertion of four miR124T sequence in a VSV-G pseudotyped LV (VSV/LV-PGK-LacZ-miR124T) significantly decreases transgene expression levels and the number of β-galactosidase-positive neurons in the striatum of adult mice (Colin et al., 2009). This detargeting approach was used to shift the tropism of LV toward astrocytes. Double-immunofluorescence staining with neuronal and astrocytic markers demonstrated that combining mokola pseudotyping and miR124T (MOK/LV-PGK-LacZ-miR124T) resulted in a transgene expression that was almost exclusively restricted to astrocytes, with 89 ± 3% β-galactosidase-S100β-positive cells and 6 ± 4% NeuN-positive cells. This effect was not restricted to the striatum as similar results were obtained in the hippocampus and cerebellum.

In conclusion, the use of these three different strategies (modulation of viral vector entry, transcription and post-transcriptional regulations) has enabled the development of efficient gene transfer systems to specifically target astrocytes (Figure 3). Thanks to the unique features of these new viral vectors, it has already been possible to make significant advances in two areas of research related to the development of innovative therapies and the modeling of neurological disorders.

**VIRAL VECTORS TARGETING ASTROCYTES: APPLICATIONS FOR BRAIN DISEASES**

**MODELING BRAIN DISEASES**

There is evidence to support the idea that the mechanisms responsible for selective neurodegeneration in some brain disorders are non-cell autonomous and based upon pathological cell–cell interactions. The selective death of the neuronal population at risk in each disorder can be better explained by the convergence of multiple pathogenic mechanisms which provoke damage within the vulnerable neuron and neighboring cell types rather than by autonomous cell mechanisms (Ilieva et al., 2009).

In order to dissect out the specific role of different cell populations in vivo (neurons, astrocytes, microglia), two different strategies were recently used. The first one relies on the use of the Cre/loxP system to silence the expression of the mutant protein in specific cell types by crossing different Cre-expressing transgenic mice with transgenic mice expressing the mutant protein flanked by loxP sites in all cell types. The opposite strategy consists of selectively expressing the mutant protein in specific cell types using either specific promoters such as GFAP or by crossing different Cre-expressing transgenic mice expressing the mutant protein after a STOP cassette flanked by loxP sites.

These two strategies were useful in providing evidence that astrocytes play a key role in the pathogenesis of ALS (Ilieva et al.,...
Viral-mediated gene therapy aiming to reduce glial proliferation is sufficient to alter the glial glutamate transport capacity early in the disease process and may contribute to pathogenesis of HD.

Glioblastoma multiforme
Glioblastoma multiform (GBM) is the most common primary tumor developing in the brain from astrocytes. Due to the quick proliferation and its infiltrative nature, complete ablation by surgery is almost impossible. The prognosis is very poor, with a median survival of 14.6–19.6 months and an inevitable relapse within a few months after the resection (Grossman et al., 2010). Indeed, GBM is a good candidate for gene therapy because tumor developing in the brain from astrocytes. Due to the quick proliferation and its infiltrative nature, complete ablation by surgery is almost impossible. The prognosis is very poor, with a median survival of 14.6–19.6 months and an inevitable relapse within a few months after the resection (Grossman et al., 2010). GBM represents, therefore, an alternative therapy (Murphy and Rabkin, 2004). In addition, replicative adenoviral vectors expressing therapeutic genes were used to mediate tumoral cells destruction. The candidate genes are inserted in the E3 deleted region and a CAR-independent entry mechanism enhancing the transduction efficiency of tumoral cells has been proposed for these new generation oncolytic viruses. To favor replication in GFAP-positive cells, three copies of glial specific & enhancer were added on the gfa2 promoter (gfa2R3), leading to a decreased growth of glioma cells (Horst et al., 2007).

Gene Therapy for Neurodegenerative Disorders
Degeneration of the nigro-striatal projection represents the major pathological hallmark of PD. Preclinical rodent and non-human primate models demonstrated a strong protective effect of glial cell line-derived neurotrophic factor (GDNF) on the nigrostriatal dopaminergic system (Gash et al., 1996; Kirik et al., 2000). However, the expression of neurotrophic factors in neurons may lead to the blockade of cellular replication (Boivin et al., 1993). But the low transduction efficiency neither improved tumor progression nor the overall survival time (Ram et al., 1993, 1994; Ganzburg et al., 1993). To improve the efficacy of the treatment, vector-producing cells (VPC) releasing MIV particles expressing the TK suicide gene) were injected into the brain after surgical resection of the tumor. However, no significant decrease of tumor mass occurred despite the bystander effect (Ram et al., 1997; Klawans et al., 1998; Shand et al., 1999; Packer et al., 2000; Rainov, 2000; Martinet et al., 2003). As an alternative therapy, Ad-TK was administered directly to GBM patients but the phase III trial showed no positive outcome (Cortin et al., 2011). Interestingly, it was shown that the preferential transduction of gloma cells is not dependent on the expression of known Ad receptors on tumor cells (Candolfi et al., 2006). Expressing the therapeutic suicide gene under the control of a strong ubiquitous promoter in combination with an immune stimulator may increase therapeutic efficacy and prevent relapse (Candolfi et al., 2006; Ghalam Muhammed et al., 2009).

As an alternative strategy to improve the therapeutic efficacy, conditionally replicative or replicative viruses were developed. The principle of oncolytic therapy is to inject directly into the tumor cells a lytic replicative-competent cytotoxic virus, such as HSV, VSV, Ad, or retroviruses, which will induce apoptosis in proliferative cells during replication (Parker et al., 2009; Zemp et al., 2010; Castro et al., 2011; Russell et al., 2012). HSV were initially used as lytic viruses in GBM therapy (Zemp et al., 2010). However, the high worldwide HSV seropositivity limits their use in the clinic and as a consequence has led to the development of other oncolytic viruses. A deletion of E1B region on Ad genome (Ad-ONYX-15) was introduced to favor apoptosis in infected gloma cells but the efficiency of this approach was too low to reach a phase II of clinical trial (Moran, 1999; Chiocca et al., 2004). In addition, replicative adenoviral vectors expressing therapeutic genes were used to mediate tumoral cells destruction. The candidate genes are inserted in the E3 deleted region and a CAR-independent entry mechanism enhancing the transduction efficiency of tumoral cells has been proposed for these new generation oncolytic viruses. To favor replication in GFAP-positive cells, three copies of glial specific & enhancer were added on the gfa2 promoter (gfa2R3), leading to a decreased growth of glioma cells (Horst et al., 2007).

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GDNF under the expression of GFAP) (Drinck et al., 2012) demonstrated the same efficacy as neuron-derived GDNF. In terms of safety, unilateral striatal GDNF expression in astrocytes did not result in delivery of bioactive GDNF to the contralateral hemispheres (potential off-target sites) as was the case when GDNF was expressed in neurons. This suggests that astrocytic neurotrophic factor expression achieved to the viral vector can be considered an efficient alternative to current gene therapeutic strategies.

Astrocytic activation, characterized by hypertrophic somata and processes, is an early hallmark in most neurodegenerative conditions. The functional impact of this activation on the progression of these diseases is still elusive and their therapeutic potential is yet unexploited. A recent study has taken advantage of the strong astrocytic tropism of AAV2/5 expressing the astrocyte-specific promoter Glf2 to test the potential of astrocyte-targeted therapies in an intact animal model of Alzheimer’s disease (AD) (Furman et al., 2012). It was shown that the bilateral administration of AAV2/5 Glf2-VIVIT (a synthetic peptide that blocks the calciuneurin (CN)/nuclear factor of activated T cells (NFAT) pathway which regulates several components of the activated astrocyte phenotype) into the hippocampus of 7- to 8-month-old APP/PS1 mice, was associated with reduced glial activation, lower amyloid levels, improved synaptic plasticity, and an improved cognitive function at 16–17 months of age. This result represents a proof-of-principle that astrocytes can be considered as significant therapeutic targets not only in AD but also for other neurodegenerative diseases. Because of its specificity, lack of toxicity and capacity for widespread and long-lasting transgene expression, AAV appears to be an ideal vehicle for directing therapeutics to astrocytes.

CONCLUSION AND PERSPECTIVES

The growing importance of astrocytes in crucial brain functions and also their functions has led to the development of new genetic tools to label and manipulate these glial cells en vivo. Thanks to these tools that include targeted transgenesis and viral transduction, considerable advances were made in the understanding of astroglial biology. This first generation of astrocytic viral vectors was instrumental to start depicting their role in specific brain regions of different species. However, a better determination of these tools that include targeted transgenesis and viral transduction, considerable advances were made in the understanding of astroglial biology. This first generation of astrocytic viral vectors was instrumental to start depicting their role in specific brain regions of different species. However, a better determination of these functional elements (Wang and Wang, 2006). Clinical observations in patients suffering from neurological pathologies following viral infections suggest that other viruses could have a cerebrotropism (e.g., alphaviruses or arboviruses, Das et al., 2010; Walker et al., 2012). This illustrates the need for multidisciplinary programs that would share the expertise of neurobiologists, virologists, geneticists, and clinicians in order to overcome the limitations of current vectors and discover innovative gene transfer systems. Considering how much more might be discovered about the functions of normal or diseased astrocytes, it is tempting to suggest that we are just at the beginning of the development of astroglial viral vectors.

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