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Brief Communication

Surface modification via strain-promoted click reaction facilitates targeted lentiviral transduction

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ABSTRACT

As a result of their ability to integrate into the genome of both dividing and non-dividing cells, lentiviruses have emerged as a promising vector for gene delivery. Targeted gene transduction of specific cells and tissues by lentiviral vectors has been a major goal, which has proven difficult to achieve. We report a novel targeting protocol that relies on the chemoselective attachment of cancer specific ligands to unnatural glycans on lentiviral surfaces. This strategy exhibits minimal perturbation on virus physiology and demonstrates remarkable flexibility. It allows for targeting but can be more broadly useful with applications such as vector purification and immunomodulation.

Introduction

Over the past two decades, lentiviral vectors have been used widely as a gene delivery system for both therapeutic and basic biological applications (Dropulic, 2011; Emeagi et al., 2013; Sakuma et al., 2012), largely due to their ability to mediate stable, long term transgene expression by integrating into the genome of both dividing and non-dividing cells. Compared to their counterpart γ-retroviruses, lentiviruses are less prone to insertional mutagenesis (Montini et al., 2009,2006), capable of accommodating larger genes (De Meyer et al., 2006), and have the unique advantage of transducing target cells without complete cell division (Naldini, 1998; Naldini et al., 1996), a feature especially suited for resting and differentiated cells such as hematopoietic stem cells (Wagemaker, 2014), macrophages (Leyva et al., 2011) and neurons (Hutson et al., 2014). With demonstrable clinical safety (Liechtenstein et al., 2013), these vectors present an attractive alternative therapy for a variety of genetic diseases (Cartier et al., 2009; Cavazzana-Calvo et al., 2010), cancer (Kalos et al., 2011) as well as infectious diseases (Coutant et al., 2012; Tebas et al., 2013). In addition to the introduction of complementary therapeutic genes into target cells, lentiviral victors have been developed to induce gene silencing by siRNAs (Hutson et al., 2014; Manjunath et al., 2009; Sumimoto and Kawakami, 2007), and deliver genetic material to antigen-presenting cells for vaccination (Hu et al., 2011). In the context of laboratory and preclinical applications, lentiviral vectors have been widely used in functional genetics, cell engineering and generating animal model systems (Dropulic, 2011; Sakuma et al., 2012).

Extensive effort has been invested in the modification of the envelope glycoprotein of lentivirus to generate vectors with enhanced properties such as restricted cell tropism (Anliker et al., 2010; Lei et al., 2010; Liang et al., 2013; Morizono et al., 2009a, 2009b, 2005), easier purification (Yu and Schaffer, 2006) and reduced immunogenicity (Croyle et al., 2004; Hwang and Schaffer, 2013). Surface glycoproteins of lentivirus can be substituted by those of another virus, commonly termed pseudotyping, which is often used to alter the cell specificity of the vector or to improve particle stability and production (Cronin et al., 2005). Other methods for surface engineering of lentiviral vectors include genetic manipulation of the envelope protein sequence (Funke et al., 2008; Höfig et al., 2014; Morizono et al., 2001, 2009a, 2009b, 2005; Munch et al., 2011; Yu and Schaffer, 2006), non-covalent modification with bridging moieties such as antibodies (Morizono

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et al., 2001, 2005) or adaptor ligands (Morizono et al., 2009b), co-enveloping of virus vectors with a fusogenic protein and a membrane-bound targeting protein (Eleftheriadou et al., 2014; Lee et al., 2011; Lei et al., 2009, 2010; Yang et al., 2006), and chemical modification through the primary amino groups on surface exposed lysine residues (Croyle et al., 2004; Liang et al., 2013). Low virus titer and transductional efficiency can result from genetic modification of the lentiviral envelope, and the type of motifs that can be introduced is limited. The targeting molecules in co-enveloping strategies are limited to membrane-bound proteins (Eleftheriadou et al., 2014; Lee et al., 2011; Lei et al., 2009, 2010; Yang et al., 2006). Although the bridging adaptor approach demonstrates wide substrate scope, it requires multistep procedures and the *in vivo* stability of these complexes is debatable. Lysine acylation also allows access to a wide variety of effector moieties, however, efficiency is relatively low, specificity is problematic and the chemistry will be adversely affected by the presence of other nucleophiles in the sample. As a result, additional methods of surface functionalization that are flexible, efficient, broadly applicable, and highly specific could aid in the development of next generation lentiviral vectors.

Unlike more conventional *in vitro* bioconjugation methods, such as electrophilic modification of free lysines and cysteines, azide–alkyne cycloaddition (Agard et al., 2004; Baskin et al., 2007; Rostovtsev et al., 2002; Sletten and Bertozzi, 2008; Tornøe et al., 2002) and Staudinger ligation (Saxon and Bertozzi, 2000) reactions are highly selective, well-controlled and fast, making them preferable in complex biological systems. Metabolic incorporation of unnatural substrates bearing azide or terminal alkyne functionalities has been utilized to label glycans (Chang et al., 2009; Laughlin and Bertozzi, 2007), lipids (Kho et al., 2004), DNA (Neef and Luedtke, 2011) and proteins (Dieterich et al., 2006). Bertozzi and coworkers have demonstrated the metabolic incorporation of an azido sialic acid analog, *N*-azidoacetyl sialic acid (SiaNAz), onto cell surface glycoproteins as a chemical reporter for both *in vitro* and *in vivo* studies (Agard et al., 2004; Luchansky et al., 2003; Prescher et al., 2004; Saxon and Bertozzi, 2000). As the envelopes of lentiviruses are derived from portions of the host cell membranes, with the viral glycoproteins generated by the cellular biosynthetic machinery, we hypothesized that sialylated viral glycoproteins could be labeled through the same strategy used to label cellular proteins (Fig. 1A). As chemical "handles", the introduced azide groups are small, inert and expected to have little impact on virus assembly and function. Several azide-specific chemical reactions can be employed for subsequent modification of the viral surface, including the Staudinger ligation, Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC), and strain-promoted azide–alkyne cycloaddition (SPAAC), giving access to virtually any functionality desired. In this work the novel SPAAC reagent bicyclo[6.1.0]nonyne (BCN) featuring the combination of easy preparation, high reactivity and relatively low lipophilicity was used (Dommerholt et al., 2010) (Fig. 1A). We report here viral surface conjugation with 3 targeting ligands. One is small molecule ligand selective for prostate-specific membrane antigen (PSMA) and the other two are peptides that are known to localize in angiogenic regions. Simple and efficient SPAAC modification of viral surfaces significantly enhanced transduction toward cells bearing the targeted cell surface receptors.

Results

Metabolic incorporation of SiaNAz onto lentiviral surface

Vesicular stomatitis virus glycoprotein (VSV-G) is the most commonly used glycoprotein for pseudotyping lentiviruses due to its broad tropism, and ability to enable high-titer vector preparation (Cronin et al., 2005). As a result, VSV-G pseudotyped lentiviruses were chosen as a platform to test SiaNAz mediated surface labeling. Each pseudotyped lentivirus particle is encapsulated with approximately 216 copies of VSV-G (Croyle et al., 2004), containing two complex N-linked oligosaccharides at amino acids 179 and 336 (Puri et al., 1992). Both the extent of sialylation of the oligosaccharides and the ability of SiaNAz to replace sialic acid depend on the cell line in which the virus proteins are produced (Etchison

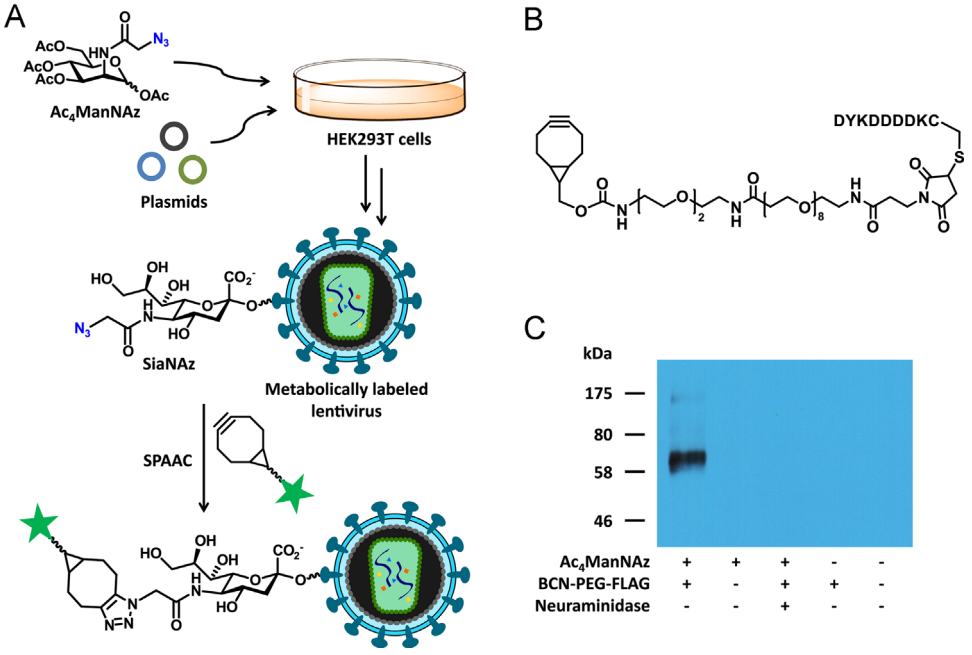

Fig. 1. Two step labeling method for surface modification of lentiviral vectors. (A) Production of lentivirus in the presence of Ac_4ManNAz leads to incorporation of SiaNAz onto viral surface glycans. Further modification via SPAAC facilitates installation of various functional molecules. (B) Structure of the reporter ligand BCN-PEG-FLAG. (C) Anti-FLAG tag western blot confirmed the incorporation of SiaNAz onto the surface of VSV-G enveloped lentivirus. The BCN-PEG-FLAG ligand was used to selectively modify SiaNAz residues on the viral surface. Prior to SPAAC modification, each virus sample was suspended in a reaction buffer (50 mM sodium citrate, pH 6.0) and incubated with neuraminidase (2.5 U/μL) or merely the buffer at 37 °C for 1 h. Each reaction sample was then run through a size-exclusion spin column equilibrated in PBS, and incubated with BCN-PEG-FLAG (300 μM) or only the buffer at room temperature for 6 h. The reaction samples were subsequently analyzed by electrophoresis on 10% SDS-polyacrylamide gels followed by western blotting with an anti-FLAG antibody. The molecular weight of VSV-G is ~67 kDa (Cannon et al., 1996).