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Versatile targeting system for lentiviral vectors involving biotinylated targeting molecules

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ABSTRACT

Conjugating certain types of lentiviral vectors with targeting ligands can redirect the vectors to specifically transduce desired cell types. However, extensive genetic and/or biochemical manipulations are required for conjugation, which hinders applications for targeting lentiviral vectors for broader research fields. We developed envelope proteins fused with biotin-binding molecules to conjugate the pseudotyped vectors with biotinylated targeting molecules by simply mixing them. The envelope proteins fused with the monomeric, but not tetrameric, biotin-binding molecules can pseudotype lentiviral vectors and be conjugated with biotinylated targeting ligands. The conjugation is stable enough to redirect lentiviral transduction in the presence of serum, indicating their potential in *in vivo* . When a signaling molecule is conjugated with the vector, the conjugation facilitates transduction and signaling in a receptor-specific manner. This simple method of ligand conjugation and ease of obtaining various types of biotinylated ligands will make targeted lentiviral transduction easily applicable to broad fields of research.

1. Introduction

Lentiviral vectors are used as a gene transduction tool in both experimental and clinical settings that require long-term transgene expression (Naldini et al., 2016). Their ability to integrate their transgenes into host chromosomes enables their transgenes to be expressed for long periods of time (Naldini et al., 1996a, 1996b). Although integration of vectors into chromosomes enables long-term transgene expression, it can also cause insertional mutagenesis, which can affect expression of specific host genes (Fraietta et al., 2018; Ranzani et al., 2013; Cavazzana-Calvo et al., 2010). Therefore, it is still important to limit integration of the vectors only to the specific target cells, which can be achieved by *ex vivo* transduction methods, including isolation of target cells, transduction of the isolated cells *in vitro*, and infusion of the transduced cells back into the body. Because hematopoietic cells can be easily isolated from the body and subsequently infused back in, ex vivo transduction by lentiviral vectors is being successfully used for transduction of hematopoietic cells, especially for treatment of monogenic hematopoietic diseases and expression of chimeric antigen receptors in current clinical trials (Naldini et al., 2016).

Long-term transgene expression is not only beneficial for treating diseases of hematopoietic cells, but also for other types of cells and tissues; for example, long-term expression of alpha-1 antitrypsin in the lungs and arylsulfatase A in the brain can be used for gene therapy of alpha-1-antitripsin deficiency and metachromatic leukodystrophy, respectively (Wilson et al., 2010; Sessa et al., 2016). Because *ex vivo* transduction of cells from solid organs is difficult, there needs to be alternative transduction approaches if lentiviral vectors are to be used to treat diseases that require long-term transgene expression.

When systemically administered, lentiviral vectors pseudotyped with commonly used envelope proteins such as vesicular stomatitis virus glycoprotein (VSV-G) are trapped by the liver and/or spleen and transduce cells in these organs, which decreases the number of vector particles available to reach the target organs (Brown et al., 2006; Morizono et al., 2005). To efficiently deliver transgenes to target cells and tissues and avoid unnecessary transduction of non-target cells in

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the liver and spleen, the vectors need to escape trapping and have the ability to specifically bind and transduce the desired cell types. Such vectors are called “targeting vectors” (Kasahara et al., 1994).

Because envelope proteins mediate binding of the pseudotypes to target cells, targeting lentiviral vectors are developed by changing the binding specificity of pseudotyping envelope proteins. This requires both eliminating their original tropisms and conferring binding affinities specific to the molecules expressed on target cells (Morizono and Chen, 2005).

The original tropisms of pseudotyping envelope proteins can usually be eliminated by mutating their receptor-binding regions and they are then used as backbones to conjugate the specific targeting ligands (Morizono and Chen, 2011; Nakamura et al., 2005). We previously succeeded in modifying the tropisms of lentiviral vectors by pseudotyping the vectors with modified Sindbis virus envelope proteins (Morizono et al., 2001, 2010, 2009a, 2009b, 2005). The Sindbis virus has two envelope proteins, E2, which mediates binding, and E1, which mediates fusion (Fields et al., 2013; Ohno et al., 1997). We mutated several receptor-binding regions of E2 to eliminate its original tropism (Dubuisson and Rice, 1993; Klimstra et al., 1999; Morizono et al., 2005). This mutated Sindbis envelope protein that lacks its natural tropism provides an ideal basis to develop a targeting lentiviral vector by conjugation with targeting ligands (Ahani et al., 2016; Aires da Silva et al., 2005; Bergman et al., 2004; Kasaraneni et al., 2017, 2018; Yang et al., 2006).

Approaches for conjugating targeting ligands are largely categorized as either covalent or non-covalent conjugation. The first involves expression of targeting ligands on the viral envelope by making fusion proteins of envelope proteins or membrane-anchoring proteins with targeting ligands. While conjugation by this method is stable, conjugation of each targeting ligand requires DNA cloning and validation of structures and expression levels (Bender et al., 2016; Funke et al., 2008; Kasahara et al., 1994; Munch et al., 2011; Nakamura et al., 2005; Sandrin et al., 2003; Somia et al., 1995). Additionally, the functions of fusion proteins must be retained for each targeting ligand, as fusion of targeting molecules sometimes affects the functions of envelope proteins and/or targeting ligands (Fielding et al., 1998). For example, fusion of murine leukemia virus envelope proteins with targeting ligands results in loss of the fusion activity of the envelope protein, which is indispensable for transduction (Zhao et al., 1999). The other method is to conjugate targeting molecules non-covalently to the vectors that have adaptor molecules on their surfaces. In this approach, once the function and expression levels of the adaptor molecule on the viral surface are validated, it is not necessary to clone expression plasmids for different types of target molecules and confirm those properties every time the targeting ligands are changed.

We previously used the ZZ peptide, IgG Fc-binding peptide derived from protein A, as an adaptor molecule fused with the mutated Sindbis virus envelope protein (2.2, Fig. 1A and B) to non-covalently conjugate targeting antibodies to lentiviral vectors. Lentiviral vectors pseudotyped with 2.2 can be easily conjugated with antibodies against various target molecules, including CD4, Transferrin receptor 1 (TfR1), PSCA, CD19, CD20, DC-SIGN, CD34, and P-glycoprotein, by simply mixing the vectors with antibodies (Liang et al., 2009a, 2009b; Morizono et al., 2001, 2010, 2006, 2005; Pariente et al., 2007). The vectors specifically transduce cell types recognized by the conjugated antibodies. Due to the ease of conjugating antibodies, this targeting lentiviral vector system has been successfully used by our research group and those of others (Anderson et al., 2009; Bergman et al., 2004; Cao et al., 2016; Lafitte et al., 2012; Wu et al., 2012; Zhang et al., 2011, 2009; Zhang and Roth, 2010). However, the conjugated antibodies are detached by competitive binding of serum antibodies to the ZZ domain when serum immunoglobulin is present (Morizono et al., 2010). Therefore, the current applications of this targeting lentiviral vector are limited to *in vitro* settings and an immunodeficient mouse model that does not have serum immunoglobulin (Liang et al., 2009a, 2009b; Morizono et al., 2005; Pariente et al., 2008, 2007). More stable conjugation methods using adaptor molecules that have higher affinity for their binding molecules need to be developed to overcome this problem.

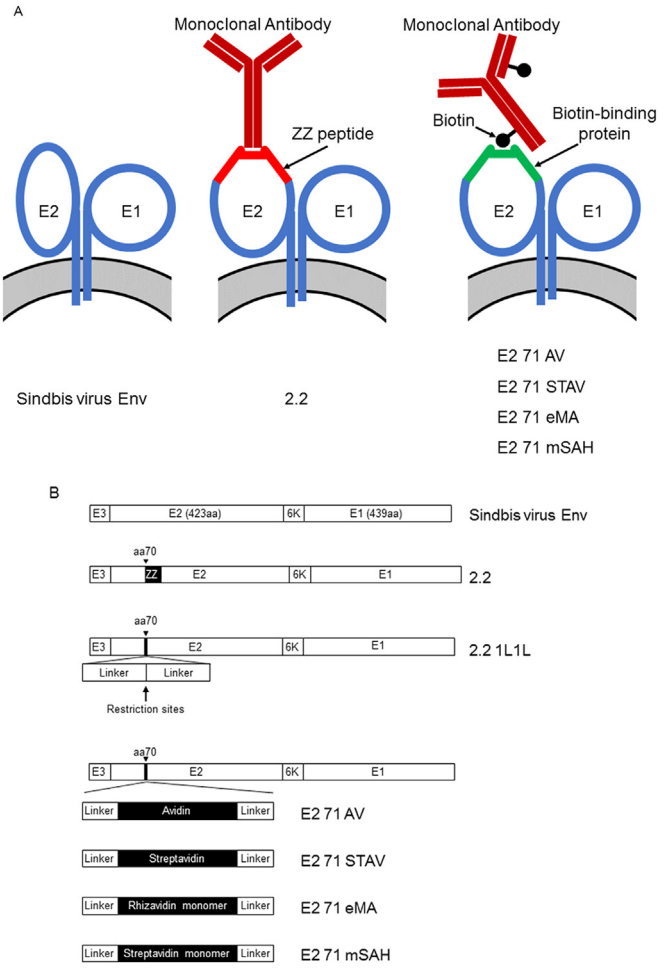

Fig. 1. Strategies to conjugate targeting antibodies to lentiviral vectors. (A) Schematic representation of conjugating targeting antibodies to lentiviral vectors. Sindbis virus envelope proteins consist of two types of envelope proteins, E2, which mediates receptor binding, and E1, which mediates fusion between the cell membrane and viral envelope. The 2.2 pseudotype contains the ZZ peptide inserted into the E2 protein. The ZZ peptide binds the Fc region of antibodies. E2 71 AV, STAV, eMA, and mSAH have avidin, streptavidin, monomeric rhizavidin, and the monomeric streptavidin/rhizavidin hybrid, respectively, in E2. These molecules are known to bind biotin; therefore, the E2 71 AV, STAV, eMA, and mSAH pseudotypes are expected to be conjugated with biotinylated antibodies. (B) The two integral membrane glycoproteins, E1 and E2, form a heterodimer and function as a unit. E3 and 6K work as signal sequence peptides for E2 and E1, respectively. 2.2 contains the ZZ peptide at aa 71 of E2, and 2.2 1L1L replaces the ZZ peptide of 2.2 with flexible linkers encompassing restriction sites for cloning. E2 71 AV, STAV, eMA, and mSAH have core sequences of avidin, streptavidin, monomeric rhizavidin, and the monomeric rhizavidin/streptavidin hybrid between the flexible linkers at aa 71 of E2.

Avidin and streptavidin are known to bind biotin at exceptionally high affinities. The dissociation constant (Kd) of binding between these two molecules is 10^{-15}, which is 10^{7-8} less than the Kd of the binding between the ZZ domain and the Fc region of antibodies (Laitinen et al., 2006). Therefore, molecules fused with them can be conjugated with biotinylated targeting ligands.

One group conjugated biotinylated antibodies on lentiviral vectors pseudotyped with both membrane-anchored avidin or streptavidin (for conjugation with biotinylated targeting ligands) and wild-type baculovirus envelope protein (for subsequent fusion) (Kaikkonen et al.,