Molecular Determinants of Vectofusin-1 and Its Derivatives for the Enhancement of Lentivirally Mediated Gene Transfer into Hematopoietic Stem/Progenitor Cells*

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Gene delivery into hCD34+ hematopoietic stem/progenitor cells (HSPCs) using human immunodeficiency virus, type 1-derived lentiviral vectors (LVs) has several promising therapeutic applications. Numerous clinical trials are currently underway. However, the efficiency, safety, and cost of LV gene therapy could be ameliorated by enhancing target cell transduction levels and reducing the amount of LV used on the cells. Several transduction enhancers already exist, such as fibronectin fragments or cationic compounds. Recently, we discovered Vectofusin-1, a new transduction enhancer, also called LAH4-A4, a short histidine-rich amphipathic peptide derived from the LAH4 family of DNA transfection agents. Vectofusin-1 enhances the infectivity of lentiviral and γ-retroviral vectors pseudotyped with various envelope glycoproteins. In this study, we compared a family of Vectofusin-1 isomers and showed that Vectofusin-1 remains the lead peptide for HSPC transduction enhancement with LVs pseudotyped with vesicular stomatitis virus glycoproteins and also with modified gibbon ape leukemia virus glycoproteins. By comparing the capacity of numerous Vectofusin-1 variants to promote the modified gibbon ape leukemia virus glycoprotein-pseudotyped lentiviral vector infectivity of HSPCs, the lysine residues on the N-terminal extremity of Vectofusin-1, a hydrophilic angle of 140° formed by the histidine residues in the Schiffer-Edmundson helical wheel representation, hydrophobic residues consisting of leucine were all found to be essential and helped to define a minimal active sequence. The data also show that the critical determinants necessary for lentiviral transduction enhancement are partially different from those necessary for efficient antibiotic or DNA transfection activity of LAH4 derivatives. In conclusion, these results help to decipher the action mechanism of Vectofusin-1 in the context of hCD34+ cell-based gene therapy.

Lentiviral vectors (LVs)§ derived from human immunodeficiency virus, type 1 (HIV-1) are becoming major tools for gene transfer. Numerous clinical trials are currently being conducted, with promising therapeutic effects for the treatment of various diseases such as immune deficiencies, anemias, cancers, neurological disorders, and HIV infection (1). Nevertheless, optimization of clinical protocols is necessary to improve the efficiency, safety, and cost of lentiviral gene therapy.

For LV applications relying on ex vivo transduction of hCD34+ hematopoietic stem/progenitor cells (HSPCs), viral vector entry represents a critical step: the adhesion and the fusion of viral particles with the plasma membrane of HSPCs (2). Viral entry efficiency is partially dependent on the envelope glycoprotein (GP) used to pseudotype LVs and, therefore, the relative paucity of viral receptors interacting with the GP of choice. Among the first and still widely used GPs for pseudotyping LVs is the vesicular stomatitis virus GP (VSV-G), which has a broad tropism, a consequence of the specific interaction with the recently identified family of low-density lipoprotein receptors (3, 4). VSV-G pseudotypes are defined as pH-dependent vectors because the fusion of viral and cellular membranes requires endosomal acidification during vector trafficking (5). LVs can also be pseudotyped efficiently with other GPs harboring a more specific hematopoietic tropism, such as the modified gibbon ape leukemia virus GP (GALVTR) (6, 7), which has been used in the clinic for treatments of severe combined immunodeficiency (8) or graft versus host disease (9). Contrary to VSV-G, GALVTR pseudotypes are described as pH-independent vectors, meaning that the viral fusion step is independent of endosomal acidification (10) and probably occurs at the cell surface or in early endosomes after interaction with the Pit-1 sodium phosphate symporter (11–13).

Despite the strong capacity of LVs to be pseudotyped with numerous GPs, a highly efficient transduction process usually

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†† The abbreviations used are: LV, Lentiviral vector; VF-1, Vectofusin-1; HIV-1, human immunodeficiency virus, type 1; HSPC, hematopoietic stem/progenitor cell; GP, glycoprotein; VSV-G, Vesicular stomatitis virus glycoprotein; GALVTR, modified gibbon ape leukemia virus glycoprotein; eGFP, enhanced GFP.
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requires the addition of culture additives to promote viral entry. Various molecules may be used, such as polymers (e.g. Polybrene (14), DEAE-dextran (15), or polyoxamers (16)), cationic lipids (e.g. Lipofectin or Lipofectamine (17, 18)), or cationic peptides/proteins (e.g. fibronectin fragments (19–21), protamine sulfate (22), prostatic acid phosphatase fragments (23), or HIV-1 gp41- and gp120-derived peptides (24, 25)). These mostly cationic additives have the capacity to neutralize membrane charges and promote viral adhesion, viral fusion (2), virus aggregation (26), and/or virus pulldown through the formation of nanofibrillar structures (27). For clinical applications, transduction protocols usually include the human fibronectin fragment CH-296 (also called Retronectin). However, the use of this material is cumbersome both practically and for precise dosage of the additive (coating step), and Retronectin-coated surfaces may reduce the yield of cells obtained after transduction (28). Therefore, the identification of safe, soluble, and easy to manipulate additives with a potent capacity to promote target cell transduction with a broad spectrum of lentiviral pseudotypes, including clinical-grade VSV-G-LVs, remains highly necessary.

Recently, we described a new transduction enhancer, Vectofusin-1 (29), also called LAH4-A4, a short histidine-rich amphipathic peptide derived from the LAH4 family of DNA transfection agents (30–33). Vectofusin-1 promotes the transduction of HSPCs and different cell lines with a broad range of lentiviral and γ-retroviral pseudotypes with no apparent cytotoxicity (29). The enhancing effects of Vectofusin-1 are comparable with those of other viral transduction enhancers, such as semen-derived enhancer of viral infection peptide or Retronectin, a human fibronectin fragment used currently in clinical settings (29). However, the critical determinants of Vectofusin-1 playing a key role in lentiviral transduction enhancement are still unknown. To investigate this question, numerous isomers and mutants of Vectofusin-1 were designed and tested for their capacity to augment lentiviral transduction of human HSPCs, a highly relevant target cell for hematopoietic gene therapy approaches. Because Vectofusin-1 is derived from a family of peptides acting as DNA transfection agents and antibiotic molecules, we also studied whether Vectofusin-1 harbors these diverse functions.

Experimental Procedures

Peptide and Reagents—The LAH4-A4/Vectofusin-1 peptide and its derivatives were produced by standard fluorenyl-methoxy-carbonyl chloride solid-phase peptide synthesis, purified by preparative reverse-phase HPLC, and analyzed by HPLC and mass spectrometry (Genecust, Dudelange, Luxembourg). 7-amino-actinomycin D and trypsin blue were obtained from Sigma-Aldrich (St-Quentin-Fallavier, France).

Cell Line Culture—HCT116 cells derived from a human colorectal carcinoma cell line (CCL-247, ATCC), HeLa cells, HuH7 cells, and HEK293T cells (34) were cultured at 37 °C, 5% CO2 in RPMI, supplemented with 10% heat-inactivated FCS (Life Technologies).

Viral Vector Production and Vector Titering—LVs were generated by transient calcium phosphate transfection of HEK293T cells with four plasmids: the gagpol (pKLgagpol) and rev (pBA.rev) expression plasmids (34), the transfer plasmid (pCCLsin.cPPT.hPGK.eGFP.WPRE), and the plasmid encoding either the VSV-G (pMDG) or the GALVTR envelope glycoprotein (pBA.GALV/Ampho-Kana) (29). After 24 h of production, raw viral supernatants were harvested, filtered (0.45 μm), and frozen at −80 °C. The purification of eGFP-expressing VSV-G-LVs, through several membrane-based and chromatographic steps, has been described previously (34). Physical particle titers were determined by measuring HIV-1 p24 capsid contents using a commercial ELISA kit (PerkinElmer Life Sciences). Infectious titers were determined on HCT116 cells using either the detection of eGFP by flow cytometry (FACSCalibur, BD Biosciences) with titers expressed as transducing units per milliliter (35) or using quantitative PCR with titers expressed as infectious genome per milliliter (34).

Culture and Transduction of Human Primary Cells—Umbilical cord blood samples were collected with informed consent after uncomplicated births at the Centre Hospitalier Sud Francailien, Evry, France, in accordance with international ethical principles and French national law (bioethics law no. 2011-814) under declaration no. DC-201-1655 to the French Ministry of Research and Higher Studies. Human CD34+ cells were isolated by immunomagnetic selection (Miltenyi Biotec, Paris, France) from the mononuclear cell fraction of umbilical cord blood samples and stored at −80 °C. After thawing, the survival rate of hCD34+ cells was evaluated using the trypan blue exclusion method. Next, preactivation of hCD34+ cells was performed overnight as described previously (2). Preactivated cells were plated in 96-well plates, and transduction was initiated by adding the desired amount of LV particles (2 × 105 infectious genome/ml) for highly purified VSV-G-LVs and 1–2 × 106 transducing units/ml for GALVTR-LVs) mixed with or without LAH4 peptide derivatives (final concentration of 12 μg/ml). Six hours post-transduction, reactions were diluted by adding differentiation medium to each well.

Peripheral blood mononuclear cells were isolated from fresh whole blood, purchased from the French Blood Establishment, using Ficoll density gradient centrifugation (Eurobio, Les Ulis, France). Next, peripheral blood mononuclear cells were stimulated with 10 μg/ml of plate-bound anti-CD3 and anti-CD28 in the presence of 100 IU/ml rIL-2 (Miltenyi Biotec). Transductions of peripheral blood mononuclear cells were performed for 6 h with GALVTR-LVs or VSV-G-LVs (2 × 106 transducing units/ml) in the absence or presence of Vectofusin-1 (12 μg/ml).

In all primary cells, cellular mortality and transduction efficiency were evaluated, respectively, by 7-amino-actinomycin D labeling and measurement of eGFP expression using flow cytometry (FACSCalibur, BD Biosciences) after 4–6 days. Cellular immunophenotyping was performed for hCD45, hCD3, and hCD19 with fluorescently labeled mAbs (BD Biosciences). Antibacterial Activity—Antibacterial activity of LAH4 derivatives was evaluated as reported previously (32). Briefly, Escherichia coli bacteria (DH5α) grown in Luria Bertani nutrient broth until mid-logarithmic phase were diluted with Luria Bertani nutrient broth to A600 0.15 and aliquoted into 96-well microtiter plates with serial dilutions of LAH4 or Vectofusin-1 peptides and citrate buffer to adjust the pH of the culture to 5.5. After incubating the plates for 6 h at 37 °C with constant shak-
ing, bacterial growth was evaluated by monitoring $A_{600}$ using a spectrophotometer.

**DNA Transfection Activity**—One microgram of pEGFP-C1 plasmid (Clontech, Saint-Germain-en-Laye, France) was complexed with the indicated concentrations of peptide in a 150 mM NaCl solution (100 µl) for 15 min at room temperature. Next, DNA complexes were added to serum-free DMEM (final volume of 0.25 ml) and transferred into a 48-well plate containing $15 \times 10^4$ cells/well. After incubation for 3 h at 37 °C, the medium was replaced with DMEM containing 10% FCS. eGFP expression was measured 48 h post-transfection using flow cytometry.

**Results**

**Design and Evaluation of Various Vectofusin-1 Isomers for Lentiviral Transduction of Hematopoietic Stem/Progenitor Cells**—Vectofusin-1, also called LAH4-A4, is characterized by the presence of two charged residues (lysine) on both extremities of the peptide sequence (Fig. 1A). Circular dichroism studies have shown that, between these lysine residues, the central core of the peptide sequence, composed of alanine, leucine, and histidine residues, has a strong propensity to form an α-helical structure, like the prototypic LAH4 peptide (30). To systematically investigate the molecular requirements for efficient transduction enhancement, Vectofusin-1 isomers were designed with different angles subtended by the two pairs of histidine residues (60°-180°) when represented as Schiffer-Edmundson wheels (Fig. 1, C and D) from positions 6–23 (36). As a second variable, the influence of the amino acid composition between the two pairs of adjacent histidine residues was evaluated. These residues either consist of alanine residues for the “LAH4-A” series (Fig. 1, A-C) and leucine residues for the “LAH4-L” series (Fig. 1, B-D), where the nomenclature directly reflects the number of alanine or leucine residues at the corresponding locations (e.g. for LAH4-L1, L1 stands for one leucine between the two pairs of adjacent histidines) (Fig. 1D). The degree of amphipathicity was quantified using the mean helical hydrophobic moment ($\mu_{H}$), calculated with the Heliquest web server (37, 38). Peptides of the LAH4-A series harbor a stronger degree of amphipathicity compared with peptides of the LAH4-L series.

The A and L isomers of Vectofusin-1 were tested for their capacity to modulate the transduction of preactivated hCD34+ HSPCs with low titers of GALVTR-LVs (Fig. 1, E and F) or VSV-G-LVs (Fig. 1, G and H). As expected, the transduction of hCD34+ HSPCs with GALVTR-LVs is highly inefficient (around 1%) in the absence of any transduction enhancers (Fig. 1, E and F). On the contrary, the transduction levels are enhanced in the presence of any members of the LAH4-A or LAH4-L series but to different extents. The strongest effect being observed for Vectofusin-1/ LAH4-A4 (Fig. 1E). The most efficient peptides on GALVTR infectivity, in either the LAH4-A or LAH4-L series, harbor a hydrophilic angle of 140° (i.e. Vectofusin-1/LAH4-A4 and LAH4-L4apo, suggesting that this feature is highly relevant. Furthermore, a high degree of amphipathicity ($\mu_{H} >0.3$) may be crucial because Vectofusin-1 and LAH4-A5 are the most potent peptides in the entire family. These data have been confirmed with dose-response curves showing that Vectofusin-1 is approximately three to four times more efficient than LAH4-L1 (Fig. 2), published previously for its capacity to enhance GALVTR-LVs infectivity (39).

Concerning HSPC transduction with highly purified VSV-G-LVs, the results were strikingly different from those obtained with GALVTR-LVs. Only two peptides significantly improved lentiviral transduction (**, $p < 0.01$); namely, Vectofusin-1/ LAH4-A4 and LAH4-L4apo, the two peptides harboring a hydrophilic angle of 140°. Surprisingly, two peptides, LAH4-A2 and LAH4-A6apo, significantly inhibited lentiviral transduction compared with the baseline in the absence of any culture additive (Fig. 1G). For all other peptides, the slight variations in lentiviral transduction efficiency were poor (*, $p < 0.05$ for LAH4-L0 and LAH4-L3) or not statistically significant.

**Critical Determinants of Vectofusin-1 for Lentiviral Transduction Enhancement**—To evaluate the specific role of Vectofusin-1 histidine residues on lentiviral transduction enhancement, Ala-scanning mutagenesis was performed (Fig. 3A). LAH2-A4 and LAH2-A6 mutants, containing only two histidine residues, harbor hydrophilic angles of 100° and 140°, respectively (Fig. 3B). Because Vectofusin-1 is highly efficient on GALVTR-LVs, subsequent experiments involving Vectofusin-1 mutants were performed on HSPCs transduced with GALVTR-LVs. As shown in Fig. 3C, the transduction of hCD34+ HSPCs with GALVTR-LVs was comparable in the presence of either LAH2-A6 or Vectofusin-1 with no apparent cytotoxicity (data not shown). On the contrary, LAH2-A4 is not functional. This loss of activity may be due to the presence of a non-optimal angle (100°) subtended by the two histidine residues (Fig. 3B). The total absence of histidine residues (K2-L10A12-K2 peptide) is also highly deleterious, with a more than 80% decrease in lentiviral transduction compared with the Vectofusin-1 condition. Therefore, it seems that histidine residues strongly improve the efficiency of Vectofusin-1 only when the hydrophilic angle subtended by the latter corresponds to 140°.

To better define the role of the lysine residues, at either the N-terminal or the C-terminal extremity of Vectofusin-1, four peptide derivatives were designed (Fig. 4A). As shown in Fig. 4B, transduction of HSPCs with GALVTR-LVs is not detectable in the presence of LAH4-A4-dKn (corresponding to a deletion of N-terminal lysine residues in Vectofusin-1). This absence of transduction is not the consequence of a strong cytotoxic effect (data not shown). The presence of only one lysine residue on the N-terminal extremity of LAH4-A4-KIn is sufficient to restore 60% of the lentiviral transduction level compared with the Vectofusin-1 control. Moreover, Vectofusin-1 activity is not improved by the addition of an extra lysine on the N-terminal extremity (LAH4-A4-K3n peptide) (Fig. 4B). Interestingly, the deletion of both C-terminal lysine residues (LAH4-A4-dKc) slightly decreased the activity to 61%, but the activity was not abrogated as observed when N-terminal lysine deletions were performed.

Next, to identify the minimal active sequence in Vectofusin-1, necessary for an efficient viral transduction enhance-
ment, shorter peptides were designed (Fig. 5A). As shown in Fig. 5B, the deletion of the C-terminal alanine (LAH4-A4-d1aa) has a tendency to decrease peptide efficiency without statistical significance. Deletion of one amino acid residue on both sides of the peptide (LAH4-A4-d2aa) decreased efficiency to 30% compared with Vectofusin-1. Finally, deletion of two amino
acid residues from the C-terminal side (LAH4-A4-d2Caa) or three amino acid residues (LAH4-A4-d3aa) or five amino acids residues (LAH4-A4-d5aa) decreased efficiency below 15% (Fig. 5B). In conclusion, any attempt to shorten Vectofusin-1 peptide length was detrimental for the promotion of lentiviral transduction of HSPCs.

**Evaluation of Vectofusin-1 Activity on Lentiviral Transduction of Human T and B Cells**—Because Vectofusin isomers act differently on the transduction of hCD34+ cells depending on the lentiviral pseudotype used, the activity of Vectofusin-1 was evaluated on B and T cells to define the influence of the primary cell type and the lentiviral pseudotype on its transduction enhancer activity. As shown in Fig. 6, Vectofusin-1 enhanced 4-fold the transduction of human B and T cells with GALVTR pseudotypes. However, in the same experiments, Vectofusin-1 was unable to promote the transduction of B and T cells using VSV-G pseudotypes. This result is reminiscent of observations with hCD34+ cells (Fig. 1), showing less efficiency of Vectofusin isomers on VSV-G-LV than with other pseudotypes. Together, these data underline the influence of cell type and the lentiviral pseudotype on its transduction enhancer activity of Vectofusin-1 derivatives.

**Evaluation of the Antibiotic Activity of Vectofusin-1**—Vectofusin-1 is a derivative of the LAH4 peptide, which has antibiotic activity (33, 40–42). This function has never been evaluated on Vectofusin-1 isomers. Using a protocol described previously (32), the antibiotic activity of Vectofusin-1 was evaluated on the DH5α E. coli strain. As shown in Fig. 7, the LAH4 control peptide strongly inhibited bacterial growth above 12 μg/ml, whereas Vectofusin-1 was not able to affect bacterial growth, even at concentrations as high as 100 μg/ml. Interestingly, LAH4 was highly inefficient for the enhancement of HSPC transduction with either GALVTR-LVs or VSV-G-LVs (data not shown). These data underline the fact that these two functions likely rely on different critical molecular determinants.
Cell Line Transfection with Vectofusin-1 Derivatives—LAH4 derivatives have been described previously as nucleic acid transfection agents (31-33, 43). Therefore, we tested the capacity of Vectofusin-1 and some derivatives to transfect 293T cells with an eGFP-expressing plasmid. As shown in Fig. 8A, 12 μg/ml of Vectofusin-1 was not sufficient to efficiently promote the transfection of 293T cells. However, an increase in Vectofusin-1 concentration to 24 μg/ml allowed highly efficient transfection of 293T cells. Next, this optimal concentration of Vectofusin-1 was used to transfect various cell lines. As shown in Fig. 8B, Vectofusin-1 is capable of promoting the transfection of all cell lines tested, although to a different extent.

Discussion

In this study, using a series of isomers and mutants, we investigated the structure-function characteristics of Vectofusin-1, also called LAH4-A4, that determine its capacity to promote transfection of 293T cells.
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FIGURE 8. DNA plasmid transfection of different cell lines using Vectofusin-1 and some derivatives. A, the eGFP-expressing plasmid was mixed with 12, 18, 24 or 36 μg/ml of Vectofusin-1. Next, the DNA/peptide mixture was diluted in 200 μl of DMEM without FCS and loaded onto cell monolayers. 3 h post-transfection, the medium was replaced with DMEM containing 10% FCS. 48 h later, transfection efficiencies were estimated by monitoring eGFP expression using flow cytometry. Data are the mean ± S.D. of two independent experiments performed in duplicate. B, HCT116, HeLa, HuH7, and 293T cells were transfected as in A in the presence of 24 μg/ml of Vectofusin-1. Data are represented as the mean ± S.E. of three independent experiments performed in duplicate. C, 293T cells were transfected as in A but in the absence (NaCl) or presence of 24 μg/ml of LAH4-L1 (n = 5), Vectofusin-1 (n = 5), LAH2-A6 (n = 3), or K2-L10-A12-K2 peptide (n = 2). Data are represented as the mean ± S.E. of n independent experiments performed in duplicate. **, p < 0.01; *, p < 0.05; Student’s t test.

lentiviral transduction in HSPCs. All Vectofusin-1 isomers tested were capable of promoting HSPC transduction with GALVTR-LVs, although to a different extent, with Vectofusin-1 being the most potent transduction enhancer. Interestingly, transduction experiments performed with the broadly used VSV-G-LVs showed slightly different results. Vectofusin-1 was still the most potent culture additive, but some Vectofusin-1 isomers were either ineffective (e.g. LAH4-L1) or capable of partially inhibiting the baseline level of HSPC transduction (e.g. LAH4-A2, LAH4-A6iso). Although Vectofusin-1 was capable of increasing 4-fold the transduction level of B cells and activated T cells using GALVTR pseudotypes or, as shown previously, using RD114TR pseudotypes (45), it was ineffective on the same cells using VSV-G pseudotypes. The GALVTR and VSV-G envelope glycoproteins lead to different viral entry pathways: a pH-independent and pH-dependent route, respectively (5, 10). This feature is likely responsible for the transduction variability observed between these lentiviral pseudotypes in the presence of Vectofusin-1 isomers. In the case of VSV-G pseudotypes, Vectofusin-1 isomers probably traffic with LVs from the plasma membrane surface (neutral pH) to acidified endosomes (below pH 6), leading to the protonation of all histidine residues. This change in the global charge of Vectofusin-1 isomers in the late endosome is accompanied by profound changes in physicochemical properties, such as their hydrophobic moment. These alterations certainly result in changes of the molecular and supramolecular structures of the peptide, similar to the transition from transmembrane to an in-plane configuration in lipid bilayers, as observed previously for the prototypic LAH4 peptide (46, 47). In contrast to the pH-independent GALVTR pseudotype, viral fusion with the plasma membrane or early endosomes occurs before endosomal acidification (10).

Overall, Vectofusin-1 and the LAH4-L4iso peptide, harboring a polar angle of 140° formed by the histidine residues in the Schiffer-Edmundson wheel representation, were the most efficient peptides in the A and L series, respectively. Furthermore, using histidine mutants of Vectofusin-1, only LAH2-A6, composed of two histidine residues with a polar angle of 140°, was still efficient on lentiviral transduction and not the LAH2-A4 harboring a polar angle of 100°. It has been shown previously that the polar angle is one of the critical parameters for amphipathic peptide activities, especially for lipid interactions and the concomitant antibacterial (48–50) and transfection activities (33). Interestingly, the K2-L10A12-K2 peptide, corresponding to Vectofusin-1, in which all histidine residues are substituted by alanine residues, was still able to promote 17% of HSPC transduction. Therefore, histidine residues strongly improve Vectofusin-1 efficacy but are not strictly necessary to promote HSPC transduction with GALVTR-LV. We have also shown the critical role of lysine residues located on the N-terminal extremity and the less important role of C-terminal lysine residues. Another parameter, the hydrophobic moment (μH), is one of the highest for the Vectofusin-1 peptide (μH 0.342) among the family of isomers. We increased this parameter by substituting all the leucine residues with isoleucine residues (i.e. LAH4-A4 peptide; μH 0.365), but it resulted in a complete loss of activity on lentiviral vectors (data not shown), suggesting that the choice of leucine as hydrophobic residue is critical. Notably, all sequences tested here that were shorter than the actual Vectofusin-1 sequence lost much of the transduction activity. It is possible that the transmembrane orientation plays a crucial role in Vectofusin-1 activity. It has been reported that an average of 21 amino acids residues is necessary to form a typical transmembrane domain in lipid bilayers (51), exactly
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the number of amino acid residues that are present between the charged lysine residues of Vectofusin-1.

In addition to its strong activity on lentiviral transduction, Vectofusin-1 is capable of promoting DNA transfection as efficiently as the LAH4-L1 prototype, but this function requires higher concentrations of peptide. Interestingly, the LAH2-A4 peptide is not capable of promoting lentiviral transduction, whereas, at the same time, it retains a high capacity to promote DNA transfection in cell lines, arguing for the fact that these two functions rely on different molecular determinants. Last but not least, Vectofusin-1 strongly promotes lentiviral transduction but has no antibiotic activity against E. coli, whereas the opposite is observed with the prototypic LAH4 peptide.

In conclusion, in the family of LAH4 histidine-rich cationic amphipathic peptides, LAH4-A4/Vectofusin-1 is the leading peptide for the promotion of viral transduction. Interestingly, the molecular requirements for the antibiotic, DNA transfection, and viral transduction functions of LAH4 derivatives seem to rely on different molecular determinants that are still to be defined.

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