Development of Sendai Virus Vectors and their Potential Applications in Gene Therapy and Regenerative Medicine

Mahito Nakanishi1,*,# and Makoto Otsu2,3,#

1Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan; 2Division of Stem Cell Therapy and 3Stem Cell Bank, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Abstract: Gene delivery/expression vectors have been used as fundamental technologies in gene therapy since the 1980s. These technologies are also being applied in regenerative medicine as tools to reprogram cell genomes to a pluripotent state and to other cell lineages. Rapid progress in these new research areas and expectations for their translation into clinical applications have facilitated the development of more sophisticated gene delivery/expression technologies. Since its isolation in 1953 in Japan, Sendai virus (SeV) has been widely used as a research tool in cell biology and in industry, but the application of SeV as a recombinant viral vector has been investigated only recently. Recombinant SeV vectors have various unique characteristics, such as low pathogenicity, powerful capacity for gene expression and a wide host range. In addition, the cytoplasmic gene expression mediated by this vector is advantageous for applications, in that chromosomal integration of exogenous genes can be undesirable. In this review, we introduce a brief historical background on the development of recombinant SeV vectors and describe their current applications in gene therapy. We also describe the application of SeV vectors in advanced nuclear reprogramming and introduce a defective and persistent SeV vector (SeVdp) optimized for such reprogramming.

Keywords: Sendai virus, gene therapy, nuclear reprogramming, induced pluripotent stem cells (iPSCs).

INTRODUCTION

Since the finding in the 1970s that cultured cells can take up nucleic acids with the aid of cationic molecules, techniques of gene delivery and expression in mammalian cells have been used widely in modern biology. Development of sophisticated gene delivery tools in the 1980s such as retroviral vectors [1, 2], adenoviral vectors [3] and cationic lipid-based reagents [4] facilitated the translation of these technologies to human gene therapy. Prototypes of the current gene delivery tools used in research and clinics were mostly established in those early periods, and then followed by significant progress in each technology [5-8].

Although various formulations of DNA–carrier complexes have been developed, recombinant viral vectors are still used as the primary choice for delivering therapeutic genes because of their efficacy. Nonetheless, the refinement of current viral vectors and development of novel viral vectors are still desired, as none of the current viral vectors satisfies all of the requirements for various applications [9, 10]. In principle, any animal virus could be tailored to form gene delivery vectors provided it can accept exogenous genes as a part of their viral genome. However, their utility is limited by various factors, including pathogenicity to humans and the availability of procedures for large-scale production. Even if candidate viruses satisfy these minimum requirements, they should have clear advantages over “classical” viral vectors in being established as practical tools. The Sendai virus (SeV) vector is a newcomer in the field with unique characteristics, making it distinct from other viral vectors.

In this review, we introduce characteristics of various SeV vectors, a unique RNA virus-based gene delivery/expression system and describe recent progress in the application of SeV vectors to molecular therapy and to advanced nuclear reprogramming.

DEVELOPMENT OF RECOMBINANT SENDAI VIRUS VECTORS

SeV (mouse parainfluenza virus type 1, hemagglutinating virus of Japan (HVJ)) is a nonsegmented negative-strand RNA virus belonging to the Paramyxovirus family [11] with a large spherical shape and an average diameter of 260 nm [12]. A SeV virion consists of the nucleocapsid (genomic RNA complexed with NP, P and L proteins), an envelope (a lipid bilayer with F and HN proteins) and a matrix (M protein) connecting the nucleocapsid and envelope (Fig. (1)).

Since its first isolation in the 1950s in Japan [13], SeV has occupied a unique position as a research tool for basic and applied biology. Long before being characterized at a molecular level, SeV particles inactivated by brief exposure to ultraviolet light or by treatment with alkylaion reagents have been widely used as research tools as a fusogenic agent to make hybrid cells [14] and as a tool for delivering macromolecules (protein and nucleic acid) into mammalian cells

*Address correspondence to these authors at the Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Central 4, Tsukuba, Ibaraki, 305-8562, Japan; Tel: +81-29-861-3040. Fax: +81-29-861-2798. E-mail: mahito-nakanishi@aist.go.jp (M.N.); and Division of Stem Cell Therapy and Stem Cell Bank, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

*These two authors contributed equally to this review article.
The complete genome sequence of the SeV Z strain was determined in 1986: first among the paramyxoviruses [19]. SeV has a 15,384-nucleotide single-strand RNA genome, consisting of six cistrons (Fig. 2). Each cistron has concise transcription initiation and termination signals and is transcribed to mRNA encoding a single polypeptide (except for the P cistron encoding P, C and V proteins; Fig. 2). This simple genome structure encouraged us to develop a recombinant SeV vector by replacing the genes dispensable for gene expression (F, HN and M genes) with therapeutic genes. However, this idea was hampered by the lack of methodology to modify the viral genome, as SeV has no DNA intermediate in its replication cycle.

Reconstitution of recombinant SeV from full-length genomic cDNA was accomplished in 1995 [20] following the establishment of a breakthrough strategy allowing the reconstitution of Rabies virus from cDNA in 1994 [21]. Since then, various SeV vectors installed with exogenous genes have been generated based on the wild-type SeV strain. In the first generation of SeV vectors, exogenous cDNA was installed between the 3' terminus and the NP gene of a full-length SeV genome [22]. These SeV vectors were replication competent and could produce large amounts of exogenous gene products when cultured in fertilized chicken eggs [23]. For medical and other practical applications, replication-defective SeV vectors with deletion in the F gene were developed subsequently [24]. These vectors were shown to induce transient but very strong gene expression so their application as tools for gene therapy and vaccine has been explored, as described below.

**APPLICATION OF SEV VECTORS IN MOLECULAR THERAPY**

At the early stage of SeV vector development, the feasibility of applying SeV vectors to various cell types was examined extensively *in vitro* and *in vivo*. SeV naturally replicates in respiratory epithelial cells and is a major pathogen causing respiratory symptoms in mice. In addition to the lung/airway epithelium [25], a recombinant SeV vector can induce strong *ex*‐gene expression in the cardiovascular system [26], in retinal epithelium [27], in hepatocytes [12], in colonic epithelium [28], in neurons [24], in dendritic cells [29] and in human hematopoietic stem cells [30]. This remarkably wide host range partly depends on the fact that the primary SeV receptor, sialic acid, is distributed universally among animal cells; the presence of a ubiquitous secondary receptor indispensable for SeV‐mediated membrane fusion has also been suggested [31]. In addition, SeV vectors rely for their gene expression only on virus‐encoded RNA polymerase and tubulin, a ubiquitous conserved cytoskeletal protein [32].

The application of SeV vectors in molecular medicine is dependent on, and is on some occasions restricted by, powerful but transient gene expression, wide host cell specificity, low pathogenicity and strong immunogenicity. To date, the feasibility for using SeV vectors clinically has been examined in the following areas: 1) as a live attenuated vaccine; 2) in gene therapy for critical limb ischemia; and 3) in cancer gene therapy.

Recombinant SeV vectors have been most intensively investigated as a vaccine platform for inducing mucosal immunity [33]. SeV was originally investigated as a xenotropic live‐attenuated vaccine as it was known to have antigenicity shared with human parainfluenza virus type 1 (hPIV‐1), an important human pathogen causing pneumonia and laryngotracheobronchitis. Results of a phase I trial where live wild‐type SeV was administered intranasally showed that SeV induced anti‐hPIV‐1 immunity effectively without any severe adverse events [34]. This result further emphasized the nonpathogenic nature of SeV to humans, partly because of the sensitivity of SeV replication to IFN [35]. Subse-
quently, replication-competent recombinant SeV vectors expressing envelope proteins of hPIV-1, hPIV-2, hPIV-3 and respiratory syncytial virus (RSV) were developed and their effectiveness was proven in model animals [36]. SeV vectors have also been investigated as platforms for vaccines against the human immunodeficiency virus [37] and influenza viruses [38].

Replication-defective (F-defective) SeV vectors expressing the angiogenic cytokine fibroblast growth factor-2 (FGF2) have been developed for treatment of critical limb ischemia [39, 40]. A phase 1/2a clinical trial using the SeV-FGF2 vector was performed in 2006 at Kyushu University (Fukuoka, Japan) and up to $5 \times 10^9$ plaque-forming units (pfu)/60 kg (body weight) of rSeV-FGF2 were administrated intramuscularly. Although the outcomes for patients remain unpublished, no severe adverse events were reported. This trial was the first to administer a recombinant SeV vector to humans directly by injection.

Applications of SeV vectors to cancer gene therapy have been investigated at the preclinical stage. In addition to the stimulation of dendritic cells with SeV vector expressing IFN (rSeV-IFN) [29], virotherapy with the unique host-restricted SeV vector rSeV/dMFct14(uPA2) (“BioKnife”) has been developed [41]. Infectivity of wild-type SeV absolutely requires the cleavage of the precursor F0 protein to F1 and F2 subunits [42], resulting in exposure of the hydrophobic N-terminus of the F1 subunit [43]. Serine protease is responsible for this cleavage in the lungs of natural hosts [44]. BioKnife is created by altering the structure of this cleavage site to that optimal to urokinase-type plasminogen activator (uPA) and by deletion of the M gene to interfere with virion production [41]. These modifications restrict the spread of this uPA-dependent recombinant SeV by membrane fusion between cells. As uPA is often activated on cancer cell surfaces and is responsible for metastasis, this virus spreads preferentially to metastatic tumors and destroys them by its intrinsic cytotoxic activity. BioKnife has been reported to be effective in treating intractable cancers such as malignant glioblastomas [45] and malignant pleural mesotheliomas [46] in animal models.

NUCLEAR REPROGRAMMING WITH SEV VECTORS

Gene delivery/expression technologies are also indispensable for reprogramming somatic cell genomes by the ectopic expression of transcription factors. The concept of nuclear reprogramming was first proposed in the 1970s, based on experiments with somatic cell fusion and was proven by the discovery of MyoD, a gene encoding a master transcription factor inducing the dynamic transition of fibroblasts to myoblasts [47]. However, no other genes encoding a master transcription factor capable of reprogramming by itself have been identified.

Reprogramming skin fibroblasts into induced pluripotent stem cells (iPSCs) by the ectopic expression of four reprogramming factors (Oct4, Sox2, Klf4 and c-Myc) opened a new era in this field [48-51]. Human iPSCs have a capacity to differentiate to all three germ layers, as do embryonic stem cells (ESCs), while tissue cells generated from iPSCs can escape from immunological rejection when transplanted to the host. In addition, using an iPSC line can avoid the ethical concern that generation of ESCs requires the destruction of normal human embryos. All of these features likely make human iPSC lines ideal sources for gene and cell therapy.

In addition to these practical aspects, the discovery of iPSCs also introduced the novel concept that cell fate conversion needs the cooperation of multiple gene products in a single cell. As genomic reprogramming is a relatively slow process, expression of the exogenous transcription factors has to be sustained for 10–20 days [52]. On the other hand, these exogenous genes should be irreversibly suppressed or—ideally—should be removed from the reprogrammed cells. This latter point is important both for avoiding unde-
sired side effects (cell transformation and insertional mutagenesis) and for maintaining full pluripotency in these cells [53].

Following the initial successes in nuclear reprogramming using gamma-retrovirus or lentivirus vectors, both of which cause provirus insertion into host genomes, researchers have made substantial efforts to establish methods for generating ex-gene-free iPSCs [53]. However, it is difficult to satisfy controversial requirements described above thoroughly with conventional gene delivery/expression tools. These and other necessities have directed the attention of researchers to the gene delivery/expression technologies once again and should facilitate the development of novel technology best suited for genomic reprogramming.

The SeV vector stands unique among other vector systems in genomic reprogramming because it can express the reprogramming genes without chromosomal integration. Wild-type SeV vectors installed with Oct4, Sox2, Klf4 and c-Myc cDNA were reported to generate ex-gene-free iPSCs, dependent on passive elimination of the genome through cell passage [54]. This prototype was replaced with a less cytotoxic backbone [55] and is now available commercially. The

![Diagram](image_url)

**Fig. (3).** Compatibility of two independent SeVdp vectors in a single cell. (Top) Structure of SeV vectors. cDNA sequences encoding for enhanced green fluorescent protein (EGFP) cDNA and Kusabira orange (KO) were installed on a single SeV vector SeVdp(KO/EGFP) (a) or on two SeV vectors SeVdp(KO) and SeVdp(EGFP) separately (b). (Middle) Fluorescence images of cells expressing KO and EGFP from these SeV vectors. Fluorescence microscopy images of KO and of EGFP were obtained separately with specific filter sets and merged after being converted to an artificial color output (green for EGFP and red for KO). Cells carry a single SeV vector SeVdp(KO/EGFP) (e) or mixture of two SeV vectors SeVdp(KO) and SeVdp(EGFP) (d). (Bottom) Expression levels of KO and EGFP were analyzed quantitatively using flow cytometry. The ratio of the signal intensities of EGFP and KO in each cell is shown as a histogram. Cells carry a single SeV vector SeVdp(KO/EGFP) (e) or mixture of two SeV vectors SeVdp(KO) and SeVdp(EGFP) (f). Reprinted from reference 26 with permission.
SeV vector with a temperature-sensitive (ts) mutation was also reported to facilitate the erasure of the vector genome [56]. However, as each of the reprogramming genes is installed on separate vectors, the balance of their expression levels is likely to vary among infected cells (Fig. (3)). Therefore, the biological characteristics of each iPSC line generated by these separate SeV vectors should be examined carefully, as the balance of expression of reprogramming genes might affect the quality of the iPSC line [57].

Defective and persistent Sendai virus (SeVdp) vectors are now also recognized as a superior tool for iPSC generation thanks to their remarkably high potential and simplicity [30]. The SeVdp vector was developed by one of us (MN) based on noncytotoxic ts mutant SeV strain clone 151 (SeV cl.151), originally isolated in 1979 [58]. Distinct from wild-type cytotoxic SeV and from other ts mutant SeV strains with defects in gene expression, SeV cl.151 is unique because it readily establishes stable persistent infection in cultured cells at 37 °C and strong expression of viral genes can be sustained indefinitely [31]. We identified the mutations responsible for this phenotype [30, 59, 60] and showed that this virus escapes from the intrinsic cytotoxicity of SeV by a defect in the induction of IFNβ production in target cells [60]. This phenotype is unique among cytoplasmic RNA viruses and is ideal for stable and reproducible gene expression.

An SeVdp vector suitable for generating ex-gene-free iPSCs (SeVdp-iPSCs) was created by deleting the M, F and HN genes dispensable for persistent gene expression and by installing four reprogramming genes instead (Fig. (4)). This “all-in-one” genome structure is essential both for certifying simultaneous expression of all the reprogramming genes at a constant ratio in a single cell (Fig. (3)), and for preventing secondary virion production from infected cells [30]. After completion of reprogramming, the SeVdp-iPS genome can be erased through suppression of viral L protein expression with short interfering RNA (siRNA) [30]. The human iPSC lines generated are ex-gene free and are quite uniform in their characteristics: more than 80% of the colonies are positive for TRA-1-60, a reliable marker for pluripotency [61] (Nishimura and Nakanishi, unpublished).

A broad target cell range is another important characteristic of SeV vector-mediated nuclear reprogramming. In addition to skin fibroblasts, SeV vectors can reprogram the nuclei of various human cells, including CD34+ cord blood cells [62], activated T lymphocytes [55] and monocytes (Nishimura and Nakanishi, unpublished). Generation of iPSC lines from peripheral blood cells, especially from nondividing monocytes, will be quite important for the practical use of tailor-made iPSCs in regenerative and molecular medicine.

FUTURE PERSPECTIVES

As we show in this review, recombinant SeV vectors are powerful tools in basic research, in molecular therapy and in regenerative medicine. Among various applications of SeV vectors, generation of human iPSCs by nuclear reprogramming is attracting broad interest and is highly valued. Although SeV vectors can generate ex-gene-free iPSCs quite efficiently compared with other gene delivery/expression systems, claims of total superiority should be treated cautiously, as “ex-gene free” is highly desirable but might not be sufficient for clinical-grade iPSC lines [53]. The long-term stability of genome structure and epigenetic conditions should be examined before the clinical application of iPSC lines generated using SeV vectors.

Since the discovery that the genomes of somatic cells could be reprogrammed to generate pluripotent iPSCs, the notion of direct genomic reprogramming of somatic cells to other cell lineages (either to terminally differentiated cells or to tissue stem cells) has also attracted significant interest [63]. Direct reprogramming (trans-differentiation) relies on the same strategy for iPSC generation, that is, ectopic expression of multiple lineage-specific protein factors in a single cell followed with culture under defined conditions optimized for each target cell type. This procedure has been investigated not only in cells cultured in vitro but also in disease target tissues in situ [64, 65]. Direct genomic reprogramming can eliminate the potential risk of tumorigenicity in iPSC-mediated tissue cells, unless the cellular life span is reset to be infinite. This approach might also accelerate the generation of target tissue cells through bypassing the time-consuming process of iPSC generation.

---

[Diagram of SeVdp Vector]

Key Structure of SeVdp Vector

1. Insertion of Gene-End Signal
2. Mutation in P gene (P517H)
3. Mutation in L gene (V981I, S1088A, C1207S, V1618L)
4. Deletion of M, F and HN genes, and Installation of exogenous genes

Fig. (4). Genome structure of defective and persistent Sendai virus (SeVdp) vector. SeVdp has mutations in the L and P genes, which are responsible for low cytotoxicity and for defective induction of IFNβ. The M, F and HN genes are deleted and replaced with genes of interest (A–D). SeVdp-iPS was installed with Oct4, Sox2, Klf4 and c-Myc cDNAs on a single vector.
Currently, direct genomic reprogramming is investigated mostly by using classical retro/lentivirus vectors. However, low reprogramming efficiency and chromosomal integration of exogenous reprogramming genes limit their translation into clinical applications. These obstacles could be overcome with the use of SeV vectors. As described above, these have the potential to deliver exogenous genes into target tissues in situ. The development of less antigenic, “smart” SeV vectors equipped with the machinery for controlling gene expression and genome stability in vivo should contribute to translation of the current direct genomic reprogramming technologies into clinical applications. Further refinement of SeV vectors through basic research is highly desired and will bring a promising future to the fields of gene therapy and regenerative medicine.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was partly supported by the Advanced Research for Medical Products Mining Program of the National Institute of Biomedical Innovation (NIBIO) (to M.N.).

REFERENCES

[1] Shimotohno K, Temin HM. Formation of infectious progeny virus after insertion of herpes simplex thymidine kinase gene into DNA of an avian retrovirus. Cell 1981; 26(1 Pt 1): 67-77.
[2] Wei CM, Gibson M, Spear PG, Scolnick EM. Construction and isolation of a transmissible retrovirus containing the src gene of Harvey murine sarcoma virus and the thymidine kinase gene of herpes simplex virus type 1. J Virol 1981; 39(3): 935-44.
[3] Berkner KL, Sharp PA. Expression of dihydrofolate reductase, and of the adjacent Elb region, in an Ad5-dihydrofolate reductase recombinant virus. Nucleic Acids Res 1984; 12(4): 1925-41.
[4] Felgner PL, Gadek TR, Holm M, Berkner KL, Sharp PA. Expression of dihydrofolate reductase, and of the adjacent Elb region, in an Ad5-dihydrofolate reductase recombinant virus. Nucleic Acids Res 1984; 12(4): 1925-41.
[5] Li HO, Zhu YF, Asakawa M, et al. A cytoplasmic RNA vector derived from nontransmissible Sendai virus with efficient gene transfer and expression. J Virol 2000; 74(14): 6545-6.
[6] Yonemitsu Y, Kitson C, Ferrari S, et al. Efficient gene transfer to the vascular system. FASEB J 2001; 15(7): 1294-6.
[7] Murakami Y, Ikeda Y, Yonemitsu Y, et al. Newly-developed Sendai virus vector for retinal gene therapy: reduction of innate immune response via deletion of all envelope-related genes. J Gene Med 2008; 10(2): 165-76.
[8] Khare R, Chen CY, Weaver EA, Barry MA. Advances and future challenges in adenoviral vector pharmacology and targeting. Curr Gene Ther 2011; 11(4): 241-38.
[9] Tiera MJ, Shi Q, Winnik FM, Fernandes JC. Polycation-based gene therapy: current knowledge and new perspectives. Curr Gene Ther 2011; 11(4): 288-306.
[10] Yi Y, Noh MJ, Lee KH. Current advances in retroviral gene therapy. Curr Gene Ther 2011; 11(3): 218-28.
[11] Kumar P, Woon-Khiong C. Optimization of lentiviral vectors generation for biomedical and clinical research purposes: contemporary trends in technology development and applications. Curr Gene Ther 2011; 11(2): 144-53.
[12] Jang JH, Schaffer DV, Shear LD. Engineering biomaterial systems to enhance viral vector gene delivery. Mol Ther 2011; 19(8): 1407-15.
[13] Warnock JN, Daigre C, Al-Rubeai M. Introduction to viral vectors. Methods Mol Biol 2011; 737: 1-25.
[14] Lamb RA, Kolakofsky D. Paramyxoviridae: The Viruses and Their Replication. Fundamental Virology, Fourth Edition 2001: 689-724.
[15] Fujita S, Eguchi A, Okabe J, et al. Sendai virus-mediated gene delivery into hepatocytes via isolated hepatic perfusion. Biol Pharm Bull 2006; 29(8): 1728-34.
[16] Kuroya M, Ishida N. Newborn virus pneumonitis (type Sendai). II. The isolation of a new virus possessing hemagglutinin activity. Yokohama Med Bull 1953; 4(4): 217-33.
[17] Okada Y. Sendai virus-induced cell fusion. Methods Enzymol 1993; 221: 18-41.
[18] Kato K, Nakanishi M, Kaneda Y, Uchida T, Okada Y. Expression of hepatitis B virus surface antigen in adult rat liver. Co-introduction of DNA and nuclear protein by a simplified liposome method. J Biol Chem 1991; 266(6): 3361-4.
[19] Uchida T, Kim J, Yamaizumi M, Miyake Y, Okada Y. Reconstitution of lipid vesicles associated with HVJ (Sendai virus) sites. Purification and some properties of vesicles containing nontoxic fragment A of diphtheria toxin. J Cell Biol 1979; 80(1): 10-20.
[20] Johnston MD. The characteristics required for a Sendai virus preparation to induce high levels of interferon in human lymphoblastroid cells. J Gen Virol 1981; 56(Pt 1): 175-84.
[21] Johnston MD, Fantes KH, Finter NB. Factors influencing production of interferon by human lymphoblastroid cells. Adv Exp Med Biol 1978; 110: 61-74.
[22] Shiota T, Iwasaki K, Shibuta H. Determination of the complete nucleotide sequence of the Sendai virus genome RNA and the predicted amino acid sequences of the F, HN and L proteins. Nucleic Acids Res 1986; 14(4): 1545-63.
[23] Shimotohno K, Temin HM. Formation of infectious progeny virus after insertion of herpes simplex thymidine kinase gene into DNA of an avian retrovirus. Cell 1981; 26(1 Pt 1): 67-77.
[24] Warnock JN, Daigre C, Al-Rubeai M. Introduction to viral vectors.
Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448(7151): 313-7.

Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131(5): 861-72.