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Efficient transduction of melanoma cells with Sendai viral vectors

Melanoma hücrelerinin Sendai viral vektörleri ile verimli transdüksiyonu

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ABSTRACT

Objective: Various viral vectors have been developed in order to delivery genes to living cells. Sendai virus (SeV) vectors are important viral vectors due to their properties suitable for gene delivery including transient gene expression, wide host cell specificity, low pathogenicity and strong immunogenicity. SeVs vectorss are highly used in molecular medicine in gene therapy, vaccine technology and regenerative.

Methods: It was evaluated the gene delivery efficiency of SeV particles in various melanoma cell lines by using fluorescence microscope and confocal laser scanning microscope imaging techniques. A375, MDA-MB-435, G361 and WM115 cells have been transduced with SeV vectors expressing green fluorescent protein (GFP) at different multiplicity of infections (MOI): 1, 3, and 9. GFP expression was checked at 24 and 48 hours later following transduction. Confocal laser scanning microscopy imaging was calculated to gene delivery efficiency.

Results: It was showed that A375, MDA-MB-435, G361 and WM115 cells are efficiently tranduced by seV even at low virus concentration with fluorescence microscopy imaging. GFP reporter gene activity started to be observed in 24 hours and peaked in 48 hours following viral transduction. Slight toxicity was observed

ÖZET

Amaç: Yaşayan hücrelere gen salımı yapmak üzere pek çok viral vektör geliştirilmiştir. Sendai viral (SeV) vektörleri geçici gen ifadesi, geniş konak özgüllüğü, düşük patojenite ve yüksek immünojenite gibi özellikleri sayesinde gen aktarımı için önemli vektörlerdir. SeV vektörleri gen tedavisi, aşı teknolojileri ve rejeneratif amaçlı moleküler tıpta sıklıkla kullanılır.

Yöntem: Bu çalışmada, farklı melanoma hücre dizilerinde SeV vektörlerinin gen aktarım verimlilikleri floresan mikroskop ve konfokal lazer taramalı mikroskop görüntüleme teknikleri ile değerlendirilmiştir. A375, MDA- MB- 435, G361 ve WM115 hücreleri yeşil flüoresan proteini (GFP) ifade eden SeV vektörleri tarafından farklı virüs derişimlerinde (enfeksiyon çarpanı (MOI): 1, 3 ve 9) transdükte edilmiştir. GFP ifadesi virüs inkübasyonundan 24 ve 48 saat sonrasında kontrol edilmiştir. Konfokal lazer taramalı mikroskop görüntüleme ile gen salım verimliliği hesaplanmıştır.

Bulgular: Floresan mikroskop görüntüleme ile düşük virüs derişimlerinde dahi (enfeksiyon çarpanı: 1), A375, MDA-MB- 435, G361 ve WM115 hücrelerinin SeV tarafından verimli şekilde transdükte edildiği gösterilmiştir. Viral transdüksiyonu takiben, GFP kontrol gen aktivitesi 24 saat içerisinde gözlemlenmeye başlanmış ve 48 saatte artış göstermiştir. Transdüksiyondan 24 saat sonrasında

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following viral transduction in all cell 24 hours later; however, cells recovered and proliferated resulting in efficient gene expression 48 hours later. According to the confocal laser scanning microscopy imaging, more than 80% of all cell lines expressed GFP 48 hours after viral transduction.

Conclusion: In conclusion, SeV vectors successfully transduced and expressed GFP reporter gene in various melanoma cell lines with high efficiency. This study discovered the use of SeV vectors in melanoma-originated cells and it can open up wide range of studies involving SeV vectors in cancer therapy and cellular reprogramming fields.

Key Words: Melanoma, Sendai virus particles, GFP, transduction, gene delivery

hücrelerde hafif toksisite gözlemlenmiş olsa da 48 saat sonrasında hücreler toksisite etkisinden kurtularak çoğalmış ve verimli şekilde gen ifadesi göstermişlerdir. Konfokal lazer taramalı mikroskop görüntüleme sonucuna göre 48 saat sonunda tüm hücre dizilerinde hücrelerin %80'inden fazlası başarılı bir şekilde GFP genini ifade etmiştir.

Sonuç: Sonuç olarak, SeV vektörleri melanoma hücrelerini yüksek verimlilikle transdükte edip gen ifadesini sağlamıştır. Bu çalışma SeV vektörlerinin melanoma orijinli hücrelerdeki kullanımını açığa çıkarmış ve SeV vektörlerinin kullanımını içeren kanser tedavi ve hücre programlama alanındaki gelecek çalışmalarına destek sağlamıştır.

Anahtar Kelimeler: Melanoma, Sendai virüsleri, GFP, transdüksüyon, gen aktarımı

INTRODUCTION

Sendai virus (SeV) belongs to the *Paramyxoviridae* family of viruses and it is a respiratory virus of mouse and rat, classified as mouse parainfluenza virus type I. Virus particles are enveloped and 150-250 nm in diameter. Its genome is a single chain RNA (15,384 bases) in the minus sense (1). SeV enters the cells by attaching itself to the sialic acid receptor present on the host cell membrane, therefore it can transduct a variety of cell types (2). The presence of a ubiquitous secondary receptor indispensable for membrane fusion has also been suggested (3). After the activation of fusion protein by a protease, virus and host cell fusion process takes place. This is followed by genome replication and protein synthesis, and finally daughter virus particles are assembled and released to extracellular space. In addition, these vectors rely for their gene expression only on virus-encoded RNA polymerase and tubulin, a ubiquitously conserved cytoskeletal protein (4).

Lung/airway epithelium is the main target of SeV particles (5), however recombinant SeV vectors can also induce strong transgene expression in cardiovascular system (6), retinal epithelium (7), hepatocytes (8), colon epithelium (9), neurons (2), dendritic cells (10), and in human hematopoietic stem cells (11).

Thanks to their powerful but transient gene expression, wide host cell specificity, low pathogenicity and strong immunogenicity, SeVs are highly used in molecular medicine with different purposes in gene therapy, vaccine technology and regenerative medicine (12-14). Until now, the feasibility for using SeV particles clinically has been recently applied in the following areas: 1) as a live attenuated vaccine; 2) in gene therapy for critical limb ischemia; and 3) in cancer gene therapy (15).

In the present work, we aimed to investigate the gene delivery efficiency of SeV particles in various melanoma cell lines including A375, MDA-MB-435,

G361 and WM115. This study examines the use of these vectors in melanocyte-originated cells and it can open up wide range of studies involving SeV vectors in cancer therapy and cellular reprogramming fields.

MATERIAL and METHOD

Cell Lines

Human melanoma cell lines (A375, MDA-MB-435, G361 and WM115) cells were purchased from ATCC (Rockville, MD, USA). A375, MDA-MB-435 and G361 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, USA), WM115 maintained in Minimum Essential Medium (MEM, Life Technologies, USA), supplemented with 10% fetal bovine serum (FBS, Life Technologies, USA), 50 U/mL penicillin (Life Technologies, USA) , 50 µg/mL streptomycin (Life Technologies, USA), 1% L-glutamine (Life Technologies, USA) and 1% non-essential amino acids (Life Technologies, USA) at 37°C in a humidified atmosphere of 5% CO2.

Sendai Virus Transductions

A375, MDA-MB-435, G361 and WM115 melanoma cells were plated into a 24-well culture plate at a density of 1x104 cells per well and incubated at 37°C, 5% CO2, overnight. SeV vectors (CytoTuneEmGFP Sendai Fluorescence Reporter, Thermo Fisher Scientific) expressing emerald green fluorescent protein (EmGFP) were added at different multiplicity of infections (MOI): 1, 3, and 9. After 24 hours of incubation period, transduction medium was removed and cells were washed with PBS (Life Technologies, USA). Fresh complete medium was added and plates were returned to the incubator. GFP expression was analyzed via confocal laser scanning microscopy (CLSM) and fluorescence microscopy.

CLSM Imaging

Reporter gene activity was assessed in cells transduced at MOI 9 concentration at 24 and 48 h of culture. Healthy cells on 24-well plates were observed under CSLM, (Zeiss LSM 510 Meta laser scanning confocal microscope, Germany), equipped with a 30 mW argon, a 1 mW, 543 nm HeNe, and a 5 mW, 633 nm HeNe laser lines. Samples were analyzed to obtain a DIC image combined with a GFP fluorescence image. Representative images were taken at 40 x magnification.

Fluorescence Microscopy

At 48 hours of culture, cells were washed with PBS buffer and fixed with 4% formaldehyde. Samples were imaged under fluorescence microscope (EVOS FL, Thermo Fisher Scientific) in order to determine the optimum MOI concentration. Representative images were obtained using two channels (DIC-phase contrast and GFP channels) at 20 x magnification.

Image Analysis

The number of GFP positive and negative cells was analyzed in five different regions of a well (3 wells per condition and time point) by using ImageJ 1.48 software. The percentage of GFP positive cells were plotted for each condition.

RESULTS

In an attempt to determine the gene delivery efficiencies of SeV vectros in melanoma cells, various cell lines of melanoma origin were transduced with SeV particles at different MOI concentrations. GFP expression was used in order to assess the reporter gene activity. Fluorescence microscopy imaging of GFP expressing cells demonstrated that all cell lines were shown to be transduced by SeV particles at 48 hour time point (Figure 1). The fluorescent signals were both detected in nuclear and cytoplasmic regions. Furthermore, increasing the MOI concentration increased the number of cells transduced after viral incubations, as expected. Therefore, according to findings illustrated in Figure 1, we assumed that SeV vectors can be used in all studied cell lines, preferably between MOI 3 and 9.

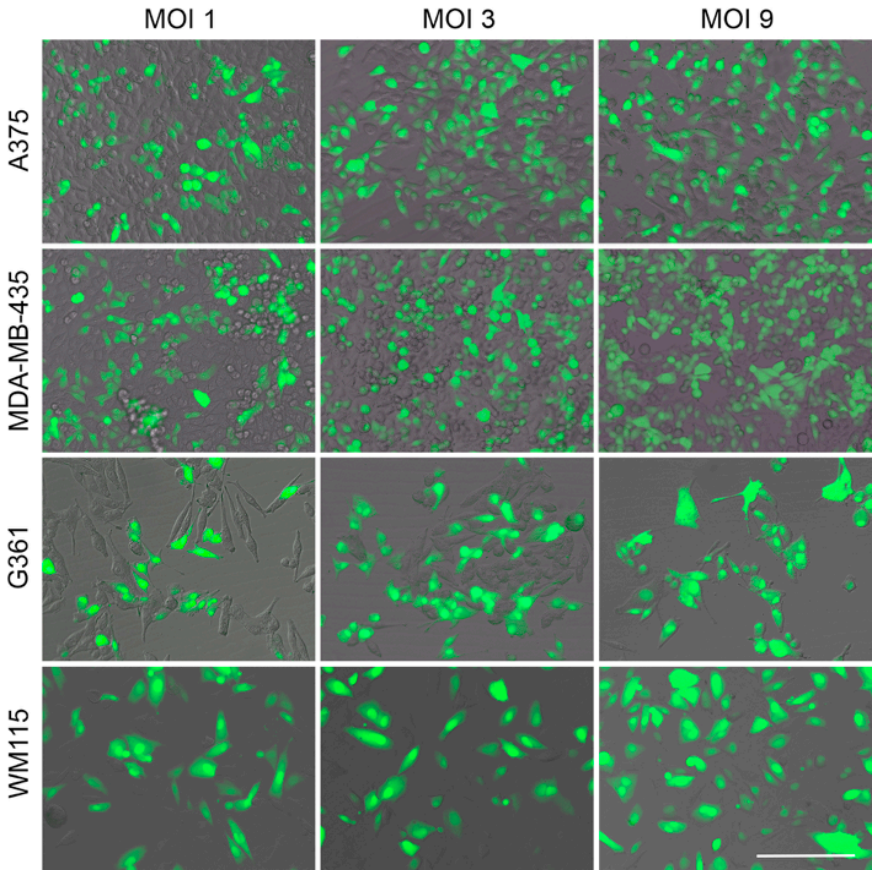

Figure 1. SeV transductions of different melanoma cell lines at different MOI concentrations. A375, MDA-MB-435, G361 and WM115 cell lines were transduced with SeV vectors expressing GFP (MOI: 1, 3 and 9) for 24 h in complete media. GFP expression (green signal) was observed after 48 h under fluorescence microscopy. Scale bar = 400μm

Transgenes carried via SeV vectors are not inserted into the host genome as in the case of lentiviral or retroviral vectors; therefore transgene expression profiles should be carefully studied. Fluorescence over 48 hour time interval was then evaluated by CLSM in order to determine the kinetics of gene expression. As shown in Figure 2, in all cell lines at MOI 9, GFP expression started as early as 24 hours and increased further at 48 hours (Figure 2). Cells observed at 48 hours showed the highest amount of fluorescence; therefore we concluded that GFP expression peaks around 48 hours following viral transduction, after which the mRNA and protein levels were started to decrease within the cells.

In order to determine whether any difference is concerned between the transduction efficiencies; the number of GFP-positive cells were analyzed by Image J. As shown in Figure 3, at 24 hours, transduction efficiency was around 40% for the A375 cells, whereas higher efficiencies were noted in other cell lines showing 60%, 70% and 80% expression efficiency in MDA-MB-435, WM115 and G361 cells, respectively (Figure 3). Furthermore, the transduction efficiency showed higher degree of variation throughout plates at 24 h time point, however after 48 h, there were more homogenous distribution of GFP-positive cells in all cell lines. After 48 h, more than 80% of all cell lines expressed GFP when analyzed by CLSM.

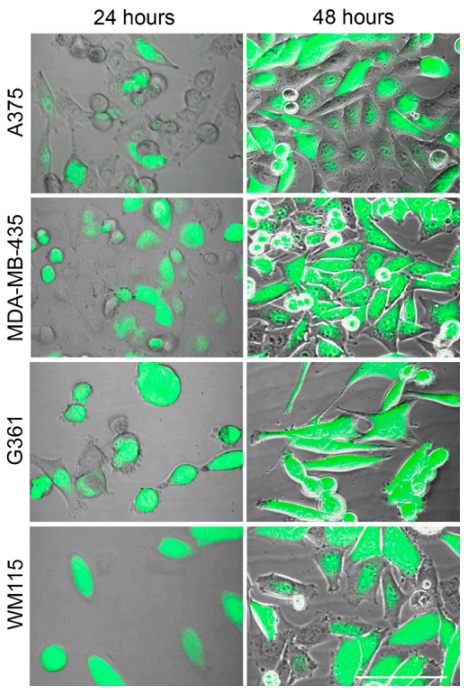

Figure 2. GFP expression over time following SeV transductions. A375, MDA-MB-435, G361 and WM115 cells were transduced with SeV vectors expressing GFP (MOI:9). GFP expression was observed by CLSM at 24 and 48 h. Scale bar = 100µm

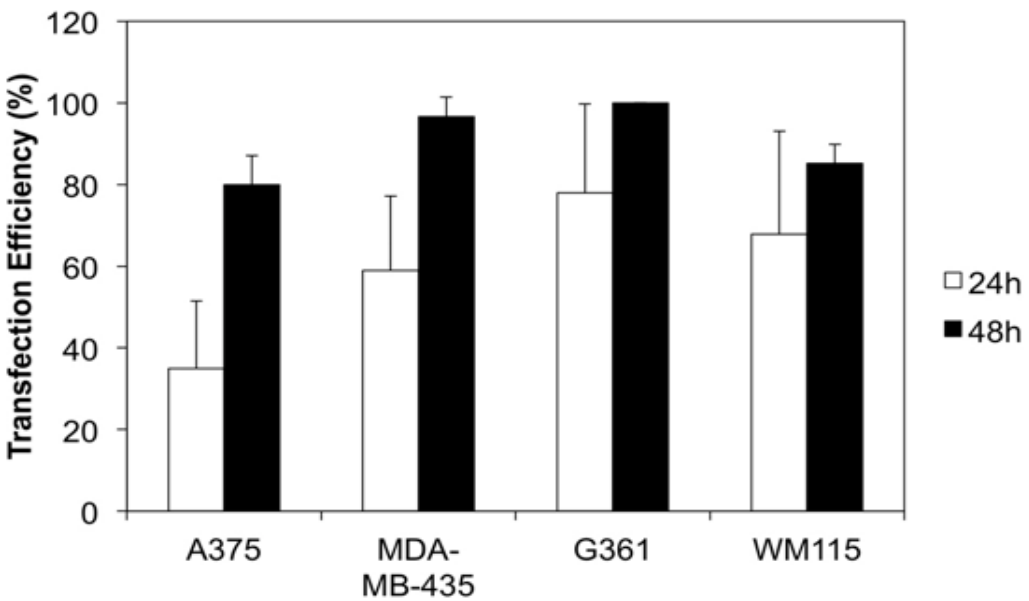

Figure 2. SeV transduction efficiencies in melanoma cell lines. A375, MDA-MB-435, G361 and WM115 cells were transduced with SeV vectors expressing GFP (MOI:9). GFP expression was imaged by CLSM and then quantified using Image J. Percentage of transduction efficiencies for all cell lines were plotted

We also observed slight toxicity at 24 h following viral transductions in all cell lines, as evident by the presence of round GFP-positive cells. As discussed by Oishi et al., this is an indication of higher degree of viral uptake (16). However, by 48 h, cells recovered and proliferated resulting in efficient gene delivery via SeV vectors.

DISCUSSION

In this study, SeV vectors successfully transduced and expressed GFP reporter gene in various melanoma cell lines with high efficiency. These results suggest that this vector has great potential for use in gene delivery and cellular reprogramming studies in cancer. For example, one of the areas where these vectors can be applied effectively is cancer cell reprogramming. In cellular reprogramming, induced pluripotent stem (iPS) cells are generated by a forced expression of reprogramming transcription factors (17). Currently, it is achieved mostly by using classical retro/lentivirus-based vectors. Unfortunately, low reprogramming efficiency and chromosomal integration of exogenous reprogramming factors limit the translation of these viral vectors into clinical settings (18). Thanks to their safer nature, SeV vectors can overcome these limitations. Until now, SeV vectors have been already used to generate iPS cells from somatic cells involving fibroblasts (19), peripheral blood mononuclear cells (20), T and B cells (21), mesenchymal stem cells (22). These studies showed that integration free iPS cells can be obtained fast and efficiently from a variety of cell types. With the help of SeV vectors, cellular reprogramming of cancer cells may also provide a powerful tool to better understand both regenerative and cancer-fate processes, with a potential to develop novel therapeutic approaches or disease modeling (23).

In conclision, we demonstrated that exogenous genes carried by SeV vectors can be efficiently expressed in various melanoma cell lines, even at low MOI concentrations. The reporter gene expression could be detected as early as 24 hours following transduction. Therefore the SeV vectors are important candidates for gene transfer to melanoma cells due to its potential for superior and safer gene delivery.

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