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# Sendai Virus-Based Oncolytic Gene Therapy

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Yosuke Morodomi, Makoto Inoue,  
Mamoru Hasegawa, Tatsuro Okamoto,  
Yoshihiko Maehara and Yoshikazu Yonemitsu

Additional information is available at the end of the chapter

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## 1. Introduction

The first gene therapy that was used on humans was performed for adenosine deaminase (ADA) deficiency in 1990 [1]. In the early days of gene therapy, it was thought to be a breakthrough in the treatment of a number of human diseases including cancer, cardiovascular disease, genetic disease, and so on. However, two severe adverse events in gene therapy served as triggers for the rethinking of the safety of gene therapy. The use of adenoviral gene therapy in an 18-year-old with an inherited enzyme deficiency at the University of Pennsylvania's Institute for Human Gene Therapy resulted in the death of the patient 4 days after the injection of the vectors into the liver in 1999 [2]. The second accident involved derived carcinogenesis that was caused by gene therapy that was performed to treat severe combined immunodeficiency-X1 in 1999 [3]. On the other hand, nonviral vectors (e.g., plasmids, liposomes, polymers, and so on.) have been developed because of these safety concerns. However, the low effectiveness of nonviral vectors in gene transduction remains a serious problem.

The key to gene therapy is safety and effectiveness. Sendai virus (SeV) vectors are able to overcome many of these problems related to gene therapy. The advantages of SeV in terms of gene therapy are the following: 1) it is nonpathogenic to human, 2) it has a high efficiency of infection, and 3) it results in high levels of gene expression.

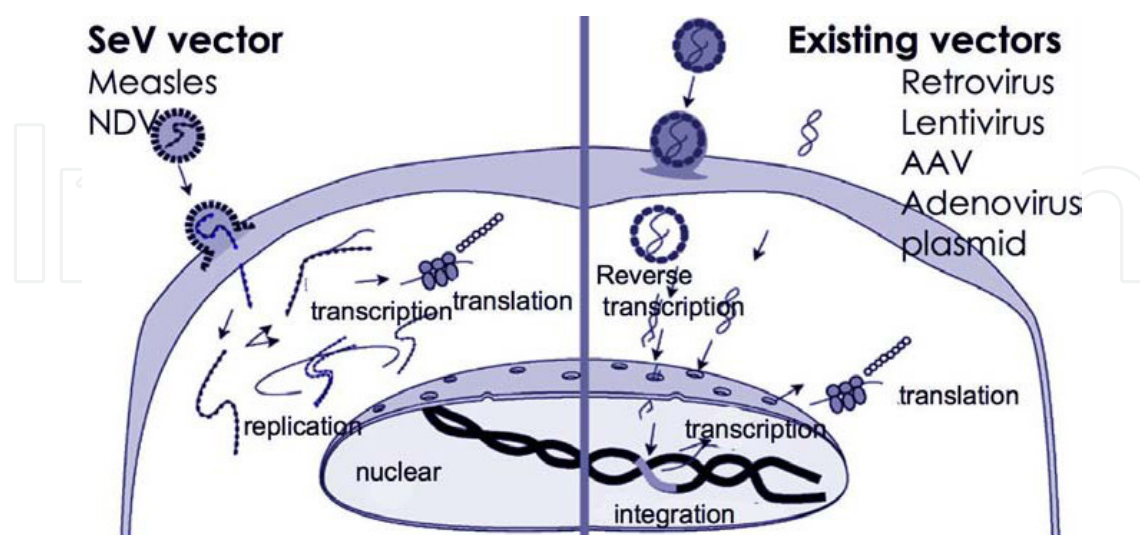
First, we would like to discuss the nonpathogenicity of SeV. The vector that is most used in gene therapy clinical trials is the Adenovirus, which is followed by the Retrovirus [4]. Adenovirus infections in humans cause pneumonia, bronchitis, croup, and so on. Retrovirus infections are one of the causes of human carcinogenesis or immunodeficiency. These vectors are pathogenic to human beings. Moreover, the infection of these vectors to human cells is associated with the risk of viral gene integration into the human genome, which contributes

to gene mutations or structural changes to chromosomes. On the other hand, SeV was originally discovered as the cause of pneumonia in rodents. Because the SeV gene exists as RNA in the cell cytoplasm throughout the life cycle of the virus from infection of the target cells to viral budding, no genetic toxicities have been confirmed (Figure 1). For these reasons, the risk of pathogenicity to humans is surprisingly low, and its safety has already been assured when it is used as a gene therapy drug.

Second, we would like to discuss the features of SeV with respect to its high efficiency of infection. Hemagglutinin-neuraminidase (HN) proteins recognize sialic acid, which is expressed as a glycoprotein or glycolipid on the cell surface. Sialic acid is widely expressed in the cells of mammals or other species, and its expression enables a large variety of SeV infections, such as those in the airway epithelium [5], saphenous vein [6], or in a variety of tumors [7,8]. In contrast, adenoviruses require the coxsackievirus–adenovirus receptor (CAR) to attach to cells, and the CAR is selectively expressed among cells, which limits its infection. In addition, adenovirus-mediated gene transfer requires a relatively long exposure time to reach maximum gene transfer efficiency, and this is a common characteristic of other currently available vectors. In contrast, SeV can infect cells in a minute or less [6]. Moreover, SeV can infect dividing cells or nondividing cells.

The third feature of SeV is its high level of gene expression. SeV has a dramatically high gene transfer efficiency compared with adenovirus vectors [6,9]. Namely, SeV can efficiently load a therapeutic gene, and gene therapy using SeV is able to decrease the amount of administration vectors in the clinical setting, resulting in a lower risk of gene therapy.

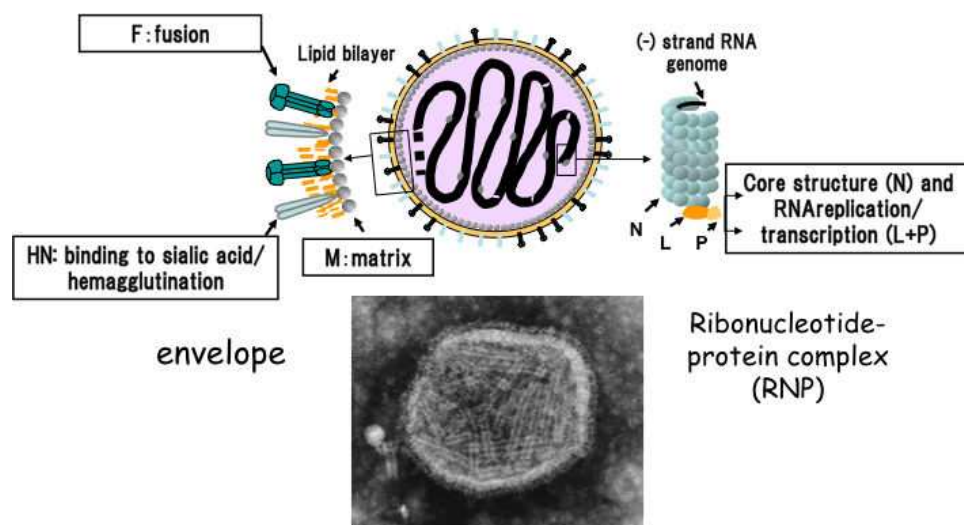
We examined whether we can apply these outstanding characteristics of SeV to the gene therapy of cancer. In this chapter, we describe the history of the investigations of oncolytic gene therapy using SeV, the present developmental status of this therapy, and the future of this therapy.



**Figure 1.** Redrawn with permission from Kinoh H et al., Front Biosci. 2008 Jan 1;13:2327-34. The life cycle of the Sendai Virus (SeV) and other vectors. SeV, Measles, and the Newcastle Disease Virus (NDV) do not result in chromosomal integration, whereas other existing vectors do. AAV, adeno-associated virus.

## 2. Structure of SeV

SeV is a negative-sense, single-stranded RNA virus of the Paramyxoviridae family. SeV genome consists of 15,384 base pairs and encodes the following 6 genes: nucleocapsid protein (N), which binds to RNA; phosphoprotein (P), which forms a small subunit of RNA polymerase; matrix protein (M), which lines the inside of viral particles; fusion protein (F), which is important for host cell penetration; HN, which is involved in the attachment to the host cells; and large protein (L), which forms a big subunit of RNA polymerase. The HN protein serves in the attachment to target cells by recognizing sialic acid on the cell surface. The F protein is cleaved through conformational change into F1 and F2, and this is triggered by local enzymatic activity, particularly that of trypsin. The cleaved F protein penetrates into the cellular membrane, which induces the membrane and the viral envelope to merge [10].



**Figure 2.** Schematic model and electron microscope photograph of SeV.

## 3. Fusogenic activity of SeV

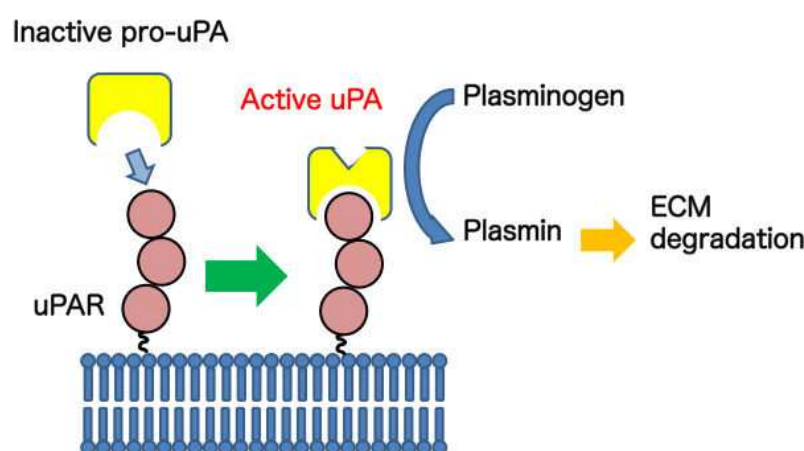
Yoshio Okada discovered the phenomenon that SeV causes the fusion of Ehrlich's tumor cells [11]. Paramyxoviridae family members including SeV have the property of cell-to-cell fusion. The fusion process, which occurs between the viral envelope and cells, may also occur between adjacent viral-infected cells when the Fusion protein is expressed on the cell surface, thus causing extensive membrane fusion and the formation of a syncytium. Cell-to-cell fusion induces apoptotic signals, resulting in cell death [12,13].

### 3.1. Oncolytic virotherapy with SeV

We have applied these fusogenic activity characteristics of SeV to cancer therapy. It is important in gene therapy for the treatment of cancer that 1) tumor-specific infections are enhanced

and 2) secondary infection is prevented. In order to obtain these properties, we modified the SeV gene by altering the F gene and deleting the M gene.

First, we would like to describe the background of the tumor-specificity abilities of Bioknife™. We focused our attention on the urokinase-type plasminogen activator (uPA). uPA is a trypsin-like serine protease that is synthesized and secreted as pro-uPA, which has little or no proteolytic activity [14]. The urokinase-type plasminogen activator receptor (uPAR) is a 55–60-kD glycoprotein that is anchored on the cell surface by a glycosyl-phosphatidylinositol linkage [15,16]. uPA binds to uPAR with high affinity. uPAR anchors uPA to the cell membrane and converts pro-uPA to active uPA, thereby localizing the proteolytic activity around the cell surface [17]. Activated uPA plays an important role in extracellular matrix degradation and results in tumor invasion and metastasis [18]. A wide variety of cancers overexpress uPAR and are associated with poor prognosis [19–23]. However, uPAR is expressed less in normal tissue except for in unusual circumstances such as inflammation [21,22,24].



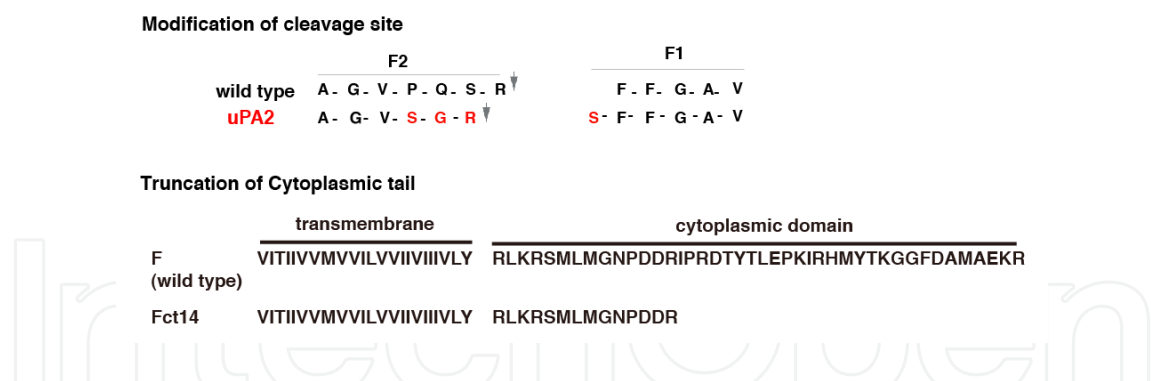
**Figure 3.** Schematic model of the urokinase activation system.

Bound and inactive pro-urokinase-type plasminogen activator (uPA) is converted to active uPA, inducing extracellular matrix (ECM) degradation. As a result, tumor invasion and metastasis are promoted.

Given that uPA activity is high around tumor cells and low around nontumor cells, we converted the F gene, which is specific to Trypsin in the wild type SeV, to a uPA-specific sequence (Figure 4). As a result, we succeeded in fusing infected cells to tumor cells only. Moreover, to optimize the fusion ability, the F gene was given an additional change, which truncated the cytoplasmic domain of the F protein (Figure 4). This genetic modification resulted in more efficient fusogenic abilities [8].

Second, we would like to describe the background of the deleting of the M gene (Figure 5). Deletion of the M gene resulted in avoidance of the budding of secondary viral particles because the M protein is indispensable for the budding of SeV. Consequently, the F proteins and HN proteins, which are expected to be the second particles in the viral spike, accumulate on the infected cell surface. If uPA is activated around the cell surface, the recombinant F

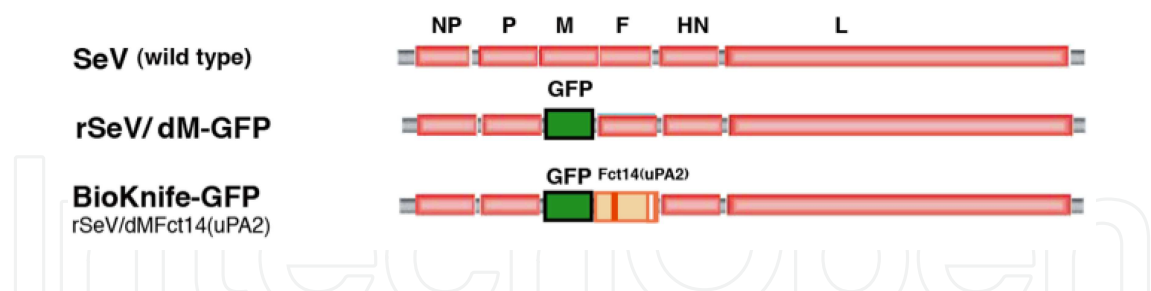




**Figure 4.** Modification of the cleavage site of the SeV-F protein, which is sensitive to the urokinase-type plasminogen activator, and truncation of the cytoplasmic tail resulted in optimization of the cell-fusion activity.

protein is cleaved, and the contiguous cells go into chain fusion reaction. These completely inhibited secondary viral particles served not only to promote fusion efficiency, but also to improve gene therapy safety.

I would like to emphasize that the oncolysis that is mediated by BioKnife™ is entirely different from conventional oncolysis. Oncolytic viruses, which is a term used to describe most viruses such as adenovirus or herpes simplex viruses, provoke the disruption of infected cells with a large number of secondary viral particles. However, the production of secondary viruses by these oncolytic viruses may limit gene therapy with respect to safety. Thus, a large number of viruses may evoke viremia and induce uncontrollable inflammatory reactions. In contrast, the oncolysis that is caused by BioKnife™ is cell death that is mediated by caspase-dependent apoptosis [13,25]. There is no need to worry about viremia, even if an explosive spread of infection is observed.

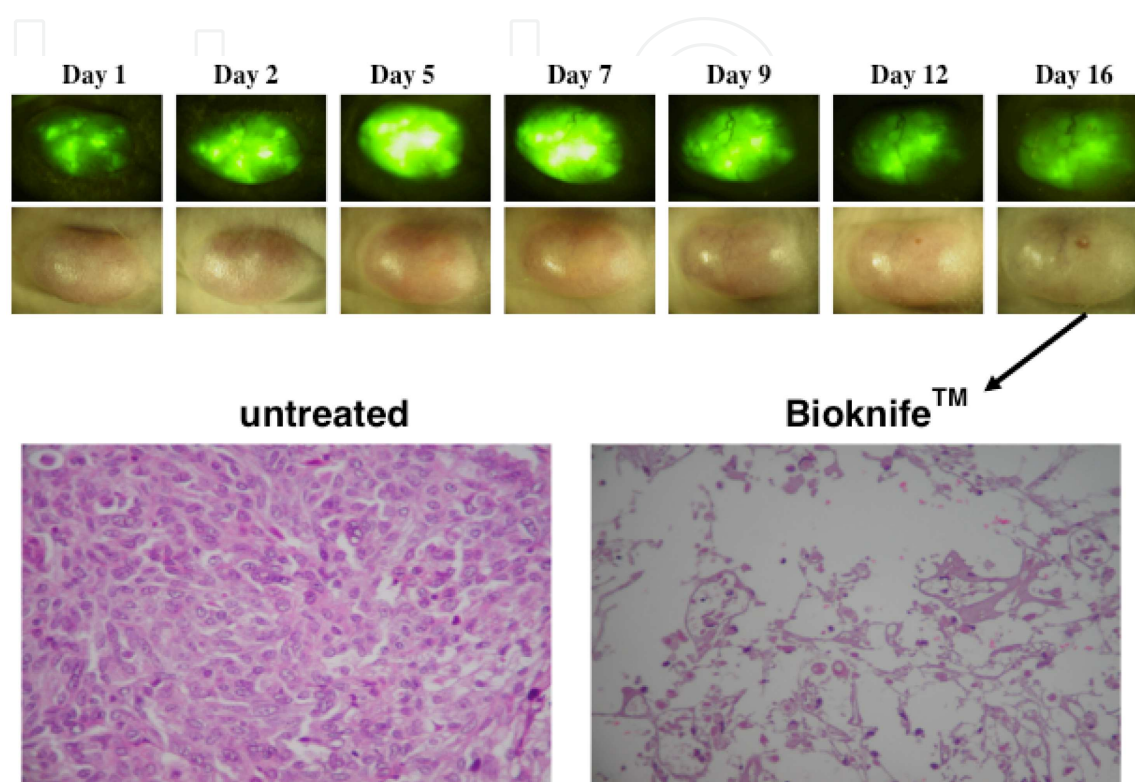


**Figure 5.** Gene structure of recombinant SeV. Wild type SeV is pictured at the top of the figure, which is followed by the M-gene deleted SeV with a substitutive load of the Green fluorescent protein (GFP) gene (rSeV/dM-GFP) in the middle. Finally, at the bottom, the F gene of rSeV/dM-GFP is transformed to a uPA-sensitive sequence (BioKnife™-GFP).

#### 4. The potential of BioKnife™

To test the cytotoxicity of Bioknife™ against tumor cells, we conducted an *in vitro* infection experiment in many types of tumor cells. Cell fusion and cell death were observed in many tumor types, and this was dependent on the uPA activity of the tumor cells. As expected,

nontumor cells were not injured [8]. Next, we tested the antitumor efficacy of BioKnife™ *in vivo*. Cells of the human prostate tumor cell line, PC3, were implanted into a nude mouse, and then BioKnife™ was injected into the tumor. BioKnife™-infected tumor presented GFP fluorescence from day 1 with the maximum GFP intensity on day 7. A microscopic examination of the subcutaneous tumor on day 16 showed that the tumor cells had been eradicated.

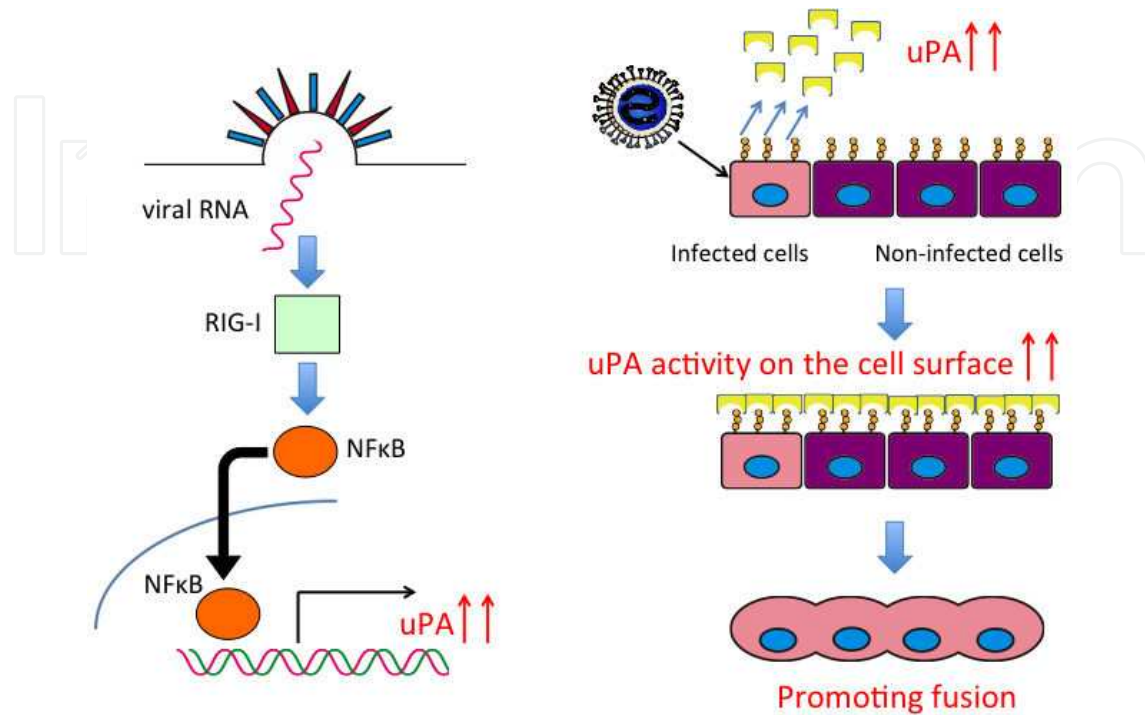


**Figure 6.** A time-course analysis of BioKnife™ infection of subcutaneously inoculated PC3 tumor cells in a nude mouse. Photomicrographs of tissue specimens on day 16 are presented in the bottom two panels.

## 5. BioKnife™ infections create a positive feedback loop of cell-to-cell fusion

The cell-to-cell fusion that is mediated by BioKnife™ provided another effect. We demonstrated that BioKnife™ infections induce simultaneous activation of the uPA expression. In addition, we found that the induction of uPA is mediated by the retinoic acid-inducible gene-1 (RIG-I), which is a viral RNA sensor that is activated by BioKnife™ infection and which activates the nuclear factor-kappa B (NF-κB) signaling pathway [25]. Activated RIG-I upregulates levels of uPA expression through the downstream protein, NFκB. Extracellularly secreted uPA binds uPAR on the tumor surface, which increases the activity of uPA. As a result, the F protein on BioKnife™-infected cells is activated and cleaved, resulting in cell fusion. It is possible that BioKnife™ results in self-induced fusion (Figure 7). This phenomenon suggests

that uPAR is necessary for the cell fusion that is mediated by BioKnife™, even if uPA is not expressed in tumor cells, and BioKnife™ infection itself facilitates the fusion activity.



**Figure 7.** A schematic model of the induction of uPA expression through Bioknife™ infection. Retinoic acid-inducible gene-1 (RIG-I) activation promotes the fusion cascade.

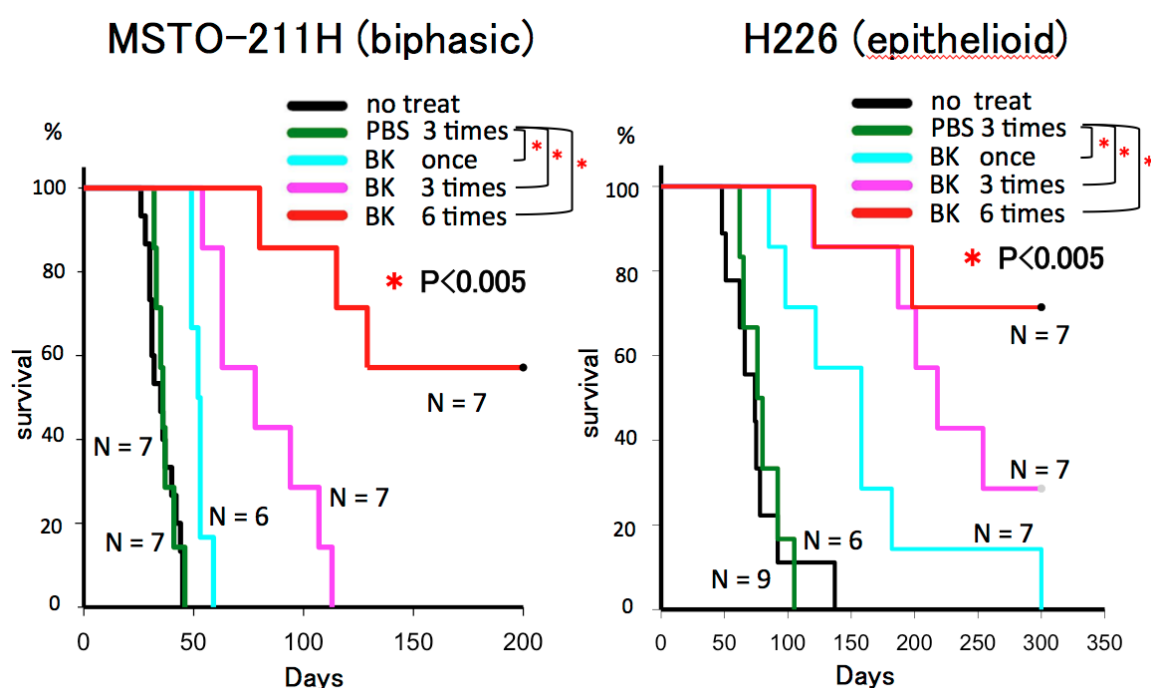
In summary, we have demonstrated the potential and the mechanisms of Bioknife™ with numerous fundamental experiments. Admittedly, Bioknife™ has no ability to infect distant tumor lesions or metastatic lesions through intravascular routes because of its instability in the blood. However, the ability of local infections of the tumor cells and the killing power are outstanding. Next, we explored diseases in which gene therapy using Bioknife™ can be applied. We examined malignant mesothelioma (MPM) in particular.

MPM is a malignancy that arises from the pleural cavity. Because MPM has a long latency period after the inhalation of asbestos [26], the number of deaths by MPM is expected to increase in the next several decades, reflecting the past usage of asbestos [27]. MPM is highly malignant due to its intractableness to treatment. Although a large number of studies have examined approaches to MPM therapy, no marked progress has appeared to overcome this disease. The median overall survival rate is less than 30 months, even if it is treated with multimodality therapy [28,29]. Thus, novel therapeutics are highly desired. MPM spreads widely throughout the pleural cavity and rarely metastasizes to distant sites in the earlier stage. In addition, MPM expresses high levels of uPAR. These characteristics suggest favorable conditions for gene therapy with Bioknife™. Thus, we explored the possibility of treatment with Bioknife™ in this disease.



## 6. Antitumor effects of Bioknife™ in a MPM orthotopic murine model

To confirm the antitumor effects in MPM, we first established two independent human orthotopic murine models. The human MPM cell lines, MSTO-211H (biphasic subtype) and H226 (epithelioid subtype), were injected into the thoracic cavity of Balb/c nu/nu mice. The tumor cells spread and formed multiple nodules in the thoracic cavity, which is similar to the pathology observed during the clinical course of human MPM. Untreated mice eventually died due to MPM progression. We assessed the performance of Bioknife™ in these MPM murine models. MPM-bearing mice were treated with BioKnife™ at the following frequencies: once, three times, or six times. The result was that, in both murine models, BioKnife™-treated cohort exhibited a significantly prolonged survival compared with the control group. The greater the number of BioKnife™ injection times, the higher the survival rate. In the group receiving 6 injections of BioKnife™, long-term survivors were observed.



**Figure 8.** Kaplan–Meier survival plot of BALB/c nude mice bearing H226 or MSTO-211H tumors that were left untreated or treated with phosphate-buffered saline or BioKnife™ in multicycle treatments. No treat indicates no treatment, PBS indicates phosphate- buffered saline treatment, and BK indicates BioKnife™ treatment.

Considering these findings, MPM is a good target for BioKnife™ treatment because the biological characteristics of MPM match the characteristics of BioKnife™. MPM spreads in the thoracic cavity and rarely develops distant metastasis. BioKnife™ can spread to adjacent tumor

cells, and local control is its primary advantage. Moreover, we would like to emphasize the accessibility of BioKnife™ in treatments of MPM. We suggest that video-assisted thoracoscopic surgery (VATS) and chest tubes are the best way to administer BioKnife™. MPM often forms nodular lesions on the pleural surface. For these targets, it is best to inject BioKnife™ intratumorally with VATS. In addition, because MPM frequently produces malignant pleural effusion [30], most cases need chest tubes. In these cases, it is convenient to administer BioKnife™ intrapleurally through the chest tube. This access route enables us to administer BioKnife™ repeatedly and safely because multiple cycles of the administration of BioKnife™ are more effective (Figure 8). Based on these results, we are planning a clinical trial using BioKnife™ to treat MPM.

## 7. BioKnife™ in the future

We described above the developmental history and the usefulness of BioKnife™. It should be noted that BioKnife™ has the ability to load other treatment genes, cytokines, tumor suppressing genes, or cancer antigens. Amazingly, the cytotoxicity of BioKnife™ depends solely on its fusion ability. In other words, there is still considerable room for improvements of this treatment modality. Moreover, there is room for further examination of the relationship between BioKnife™ and cancer immunity. Viral oncolysate is applied as a cancer vaccine in cancer immunotherapy. BioKnife™-lysed tumor cells make an extract of tumor cells. The extract contains both cancer cell proteins and virus proteins. This extract may facilitate the antigen presentation activity to dendritic cells or activate natural killer cells. Further studies are necessary to confirm this fact.

## 8. Conclusion

We developed BioKnife™, which is a uPA activity-dependent oncolytic SeV vector. This promising oncolytic vector, BioKnife™, may overcome the limitations of current gene therapy vectors. Further studies are needed to examine whether this new modality is effective in the clinical setting as a therapeutic alternative for this intractable disease.

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## Author details

Yosuke Morodomi<sup>1</sup>, Makoto Inoue<sup>2</sup>, Mamoru Hasegawa<sup>2</sup>, Tatsuro Okamoto<sup>3</sup>, Yoshihiko Maehara<sup>3</sup> and Yoshikazu Yonemitsu<sup>4</sup>

1 Department of Thoracic Oncology, National Kyushu Cancer Center, Japan

2 DNAVEC Corporation, Japan

3 Department of Surgery and Science, Graduate School of Medicine, Kyushu University, Japan

4 R&D Laboratory for Innovative Biotherapeutics, Graduate School of Pharmaceutical Sciences, Kyushu University, Japan

## References

- [1] Blaese, R. M, Culver, K. W, Miller, A. D, Carter, C. S, Fleisher, T, Clerici, M, et al. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. *Science*. (1995). Oct.;; 270(5235), 475-80.
- [2] Lehrman, S. Virus treatment questioned after gene therapy death. *Nature*. (1999). Oct.;; 401(6753), 517-8.
- [3] Hacein-bey-abina, S, Von Kalle, C, & Schmidt, M. Le Deist F, Wulffraat N, McIntyre E, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med*. (2003). Jan.;; 348(3), 255-6.
- [4] Gene Therapy Clinical Trials Worldwide [cited (2012). Jul Available from: <http://www.wiley.com/legacy/wileychi/genmed/clinical/>
- [5] Yonemitsu, Y, Kitson, C, Ferrari, S, Farley, R, Griesenbach, U, Judd, D, et al. Efficient gene transfer to airway epithelium using recombinant Sendai virus. *Nat. Biotechnol*. (2000). Sep.;; 18(9), 970-3.
- [6] Masaki, I. Recombinant Sendai virus-mediated gene transfer to vasculature: a new class of efficient gene transfer vector to the vascular system. *The FASEB Journal*. (2001). Mar. 28.
- [7] Iwadate, Y. Recombinant Sendai Virus Vector Induces Complete Remission of Established Brain Tumors through Efficient Interleukin-2 Gene Transfer in Vaccinated Rats. *Clin. Cancer Res*. (2005). May 15;; 11(10), 3821-7.
- [8] Kinoh, H, Inoue, M, Komaru, A, Ueda, Y, Hasegawa, M, & Yonemitsu, Y. Generation of optimized and urokinase-targeted oncolytic Sendai virus vectors applicable for various human malignancies. *Gene Ther [Internet]*. (2008). Nov. 27; Available from:

[http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?](http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19037241&retmode=ref&cmd=prlinks)

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- [9] Tokusumi, T, Iida, A, Hirata, T, Kato, A, Nagai, Y, & Hasegawa, M. Recombinant Sendai viruses expressing different levels of a foreign reporter gene. *Virus Res.* (2002). Jun.;86(1-2):33-8.
- [10] Takimoto, T, Taylor, G. L, Connaris, H. C, Crennell, S. J, & Portner, A. Role of the hemagglutinin-neuraminidase protein in the mechanism of paramyxovirus-cell membrane fusion. *J. Virol.* (2002). Dec.;, 76(24), 13028-33.
- [11] Okada, Y, & Tadokoro, J. Analysis of giant polynuclear cell formation caused by HVJ virus from Ehrlich's ascites tumor cells. II. Quantitative analysis of giant polynuclear cell formation. *Exp. Cell Res.* (1962). Feb.;, 26, 108-18.
- [12] Scheller, C, & Jassoy, C. Syncytium Formation Amplifies Apoptotic Signals: A New View on Apoptosis in HIV Infection in Vitro. *Virology.* (2001). Mar. 30;,, 282(1), 48-55.
- [13] Villenave, R, Touzelet, O, Thavagnanam, S, Sarlang, S, Parker, J, Skibinski, G, et al. Cytopathogenesis of Sendai Virus in Well-Differentiated Primary Pediatric Bronchial Epithelial Cells. *J. Virol.* (2010). Oct. 25;,, 84(22), 11718-28.
- [14] Petersen, L. C, Lund, L. R, Nielsen, L. S, Danø, K, & Skriver, L. One-chain urokinase-type plasminogen activator from human sarcoma cells is a proenzyme with little or no intrinsic activity. *J. Biol. Chem.* (1988). Aug. 15;,, 263(23), 11189-95.
- [15] Nielsen, L. S, Kellerman, G. M, Behrendt, N, Picone, R, Danø, K, & Blasi, F. A. Mr receptor protein for urokinase-type plasminogen activator. Identification in human tumor cell lines and partial purification. *J. Biol. Chem.* (1988). Feb. 15;,, 263(5), 2358-63.
- [16] Ploug, M, Kjalke, M, Rønne, E, Weidle, U, Høyer-hansen, G, & Danø, K. Localization of the disulfide bonds in the NH<sub>2</sub>-terminal domain of the cellular receptor for human urokinase-type plasminogen activator. A domain structure belonging to a novel superfamily of glycolipid-anchored membrane proteins. *J. Biol. Chem.* (1993). Aug. 15;,, 268(23), 17539-46.
- [17] Vassalli, J. D, Baccino, D, & Belin, D. A cellular binding site for the Mr 55,000 form of the human plasminogen activator, urokinase. *J. Cell Biol.* (1985). Jan.;, 100(1), 86-92.
- [18] Blasi, F, & Carmeliet, P. uPAR: a versatile signalling orchestrator. *Nat. Rev. Mol. Cell Biol.* (2002). Dec.;, 3(12), 932-43.
- [19] Alpar, Alpar W, Nielsen BS, Sierra R, Illemann M, Ramirez JA, Arias A, et al. Urokinase plasminogen activator receptor is expressed in invasive cells in gastric carcinomas from high- and low-risk countries. *Int. J. Cancer.* (2010). Jan. 15;,, 126(2), 405-15.
- [20] Pedersen, H, Brünner, N, Francis, D, Osterlind, K, Rønne, E, Hansen, H. H, et al. Prognostic impact of urokinase, urokinase receptor, and type 1 plasminogen activa-

- tor inhibitor in squamous and large cell lung cancer tissue. *Cancer Res.* (1994). Sep. 1; 54(17), 4671-5.
- [21] Bianchi, E, Cohen, R. L, & Thor, A. T. Todd RFIII, Mizukami IF, Lawrence DA, et al. The urokinase receptor is expressed in invasive breast cancer but not in normal breast tissue. *Cancer Res.* (1994). Feb.; 54(4), 861-6.
- [22] Li, Y, & Cozzi, P. J. Targeting uPA/uPAR in prostate cancer. *Cancer Treat. Rev.* (2007). Oct.; 33(6), 521-7.
- [23] De Bock, C. E, & Wang, Y. Clinical significance of urokinase-type plasminogen activator receptor (uPAR) expression in cancer. *Med. Res. Rev.* (2003). , 24(1), 13-39.
- [24] Eddy, A. A. Serine proteases, inhibitors and receptors in renal fibrosis. *Thromb. Haemost.* (2009). Apr.; 101(4), 656-64.
- [25] Morodomi, Y, Yano, T, Kinoh, H, Harada, Y, Saito, S, Kyuragi, R, et al. BioKnife, a uPA activity-dependent oncolytic Sendai virus, eliminates pleural spread of malignant mesothelioma via simultaneous stimulation of uPA expression. *Mol. Therapy.* (2012). Apr.; 20(4), 769-77.
- [26] Marinaccio, A, Binazzi, A, Cauzillo, G, Cavone, D, Zotti, R. D, Ferrante, P, et al. Analysis of latency time and its determinants in asbestos related malignant mesothelioma cases of the Italian register. *Eur. J. Cancer.* (2007). Dec.; 43(18), 2722-8.
- [27] Murayama, T, Takahashi, K, Natori, Y, & Kurumatani, N. Estimation of future mortality from pleural malignant mesothelioma in Japan based on an age-cohort model. *Am. J. Ind. Med.* (2005). , 49(1), 1-7.
- [28] Sugarbaker, D. J, Flores, R. M, Jaklitsch, M. T, Richards, W. G, Strauss, G. M, Corson, J. M, et al. Resection margins, extrapleural nodal status, and cell type determine post-operative long-term survival in trimodality therapy of malignant pleural mesothelioma: results in 183 patients. *J. Thorac. Cardiovasc. Surg.* (1999). Jan.;117(1):54-63-discussion, 63-5.
- [29] Bölükbas, S, Manegold, C, Eberlein, M, Bergmann, T, Fisseler-eckhoff, A, & Schirren, J. Survival after trimodality therapy for malignant pleural mesothelioma: Radical Pleurectomy, chemotherapy with Cisplatin/Pemetrexed and radiotherapy. *Lung Cancer.* Elsevier Ireland Ltd; (2011). Jan. 1; 71(1), 75-81.
- [30] Kawashima, A, & Libshitz, H. I. Malignant pleural mesothelioma: CT manifestations in 50 cases. *Am. J. Roentgenol.* (1990). Nov.; 155(5), 965-9.