

# Oncolytic Sendai virus-based virotherapy for cancer: recent advances

Kotaro Saga  
Yasufumi Kaneda

Division of Gene Therapy Science,  
Graduate School of Medicine, Osaka  
University, Osaka, Japan

**Abstract:** Many drugs have been developed and optimized for the treatment of cancer; however, it is difficult to completely cure cancer with anticancer drugs alone. Therefore, the development of new therapeutic technologies, in addition to new anticancer drugs, is necessary for more effective oncotherapy. Oncolytic viruses are one potential new anticancer strategy. Various oncolytic viruses have been developed for safe and effective oncotherapy. Recently, Sendai virus-based oncotherapy has been reported by several groups, and attention has been drawn to its unique anticancer mechanisms, which are different from those of the conventional oncolytic viruses that kill cancer cells by cancer cell-selective replication. Here, we introduce Sendai virus-based virotherapy and its anticancer mechanisms.

**Keywords:** HVJ-E, cancer therapy, apoptosis, necroptosis, anticancer immunity

## Introduction

It is generally thought that a virus is a health threat. When a virus infects host cells, it begins to increase its viral copies by replicating its genome and producing viral proteins for progeny viruses. Although host cells have several mechanisms to resist viral amplification, eg, innate immunity or apoptosis, some viruses can overcome these systems and lyse the infected cells when the viral copies reach a certain number.

Oncolytic virotherapy is based on the aforementioned viral features. Until now, many researchers have reported various oncolytic viruses that selectively replicate in cancer cells. Reovirus, Edmonston strain of measles virus, E1B-deficient adenovirus, and Newcastle disease virus are naturally discovered oncolytic viruses.<sup>1-3</sup> Other oncolytic viruses have been developed by viral genome engineering. Herpes simplex virus-1 deleting some viral genes such as  $\gamma$ 34.5, ICP6, and ICP47 has been reported as an effective virotherapeutic tool.<sup>2</sup> Moreover, armed-type oncolytic viruses having therapeutic genes, such as granulocyte-monocyte-colony stimulating factor, are more powerful for cancer treatment.<sup>4,5</sup>

As observed earlier, various oncolytic viruses have potential as safe and effective oncotherapeutics that work in a cancer cell-selective fashion. However, the mechanism of cancer cell death induction is dependent on excess intracellular viral propagation. Sendai virus has recently received attention as a new tool for virotherapy. The oncolytic mechanism of Sendai virus differs from that of conventional oncolytic viruses. Therefore, we herein introduce Sendai virus-based virotherapy.

Correspondence: Kotaro Saga  
Division of Gene Therapy Science,  
Graduate School of Medicine, Osaka  
University, 2-2 Yamada-oka, Suita, Osaka  
565-0871, Japan  
Tel +81 6 6879 3901  
Fax +81 6 6879 3909  
Email [saga@gts.med.osaka-u.ac.jp](mailto:saga@gts.med.osaka-u.ac.jp)

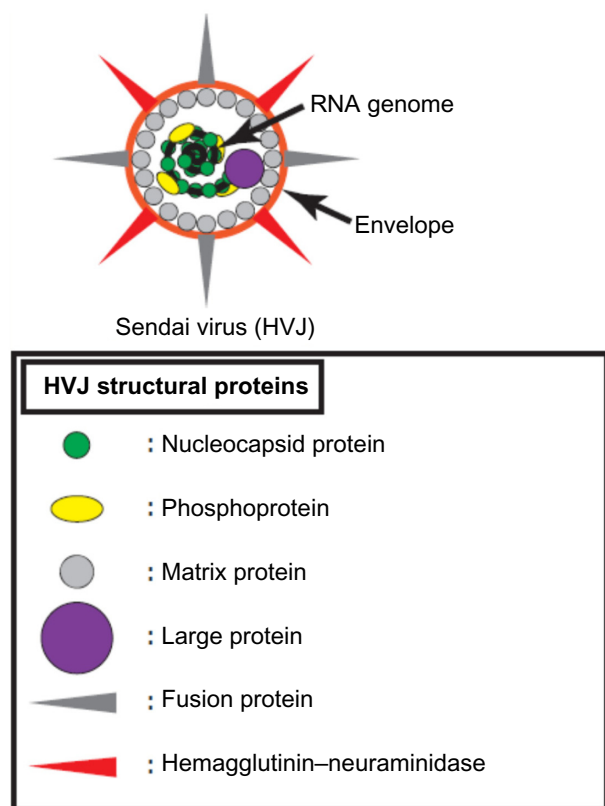
## Sendai virus

Sendai virus is a paramyxovirus and is also known as hemagglutinating virus of Japan (HVJ). HVJ has a negative-sense single-stranded RNA genome encoding the following six viral structural proteins: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin–neuraminidase (HN), and large protein (L).<sup>6</sup> N, P, M, and L form a complex with the viral genome, and the complex is covered with a host-derived envelope (Figure 1).<sup>7</sup> By contrast, HN and F are on the surface of the viral envelope (Figure 1).<sup>7</sup> HN binds to sialic acid on the surface of the host cell membrane and induces the adhesion of viral particles to the host cell surface.<sup>8</sup> F catalyzes the membrane fusion of the viral envelope with the host cell membrane and enables the viral genome to be introduced into the host cell cytoplasm for HVJ infection.<sup>9</sup> HVJ viral proteins are translated in the infected cell to generate progeny viruses. F is originally produced as the F<sub>0</sub> form without a catalytic function, and the F<sub>0</sub> form is changed to the F<sub>1</sub>/F<sub>2</sub> form, which exhibits membrane fusion activity, by the cleavage of F with a protease.<sup>10,11</sup> Human cells do not produce the protease for F activation; therefore, HVJ is unable to amplify infectious progeny viruses in humans. Therefore, few reports have demonstrated cancer therapy using HVJ as an

oncolytic virus until recently because conventional oncolytic viruses require repeated infection of cancer cells and the generation of infectious progeny for effective anticancer activity. In fact, some reports show that ultraviolet (UV)-irradiated oncolytic viruses, which lack viral reproduction potential, are unable to induce effective oncolysis.<sup>12–15</sup> However, the generation of recombinant HVJ, which specifically infects cancer cells by viral genome engineering, has recently been reported.<sup>16,17</sup> Moreover, UV-irradiated HVJ, which is known as HVJ-envelope (HVJ-E), effectively induces cancer cell death and stimulates antitumor immunity, even if HVJ-E lacks the ability to replicate. Since these developments were reported, Sendai virus-based oncolytic viruses have drawn attention for their potential use as cancer therapeutics. Here, we discuss attenuated HVJ and HVJ-E and explain the mechanism of HVJ-E-mediated anticancer activity, including the induction of apoptosis and necroptosis in cancer cells and the stimulation of antitumor immunity (Table 1).

## Genetically modified-Sendai virus

The generation of recombinant attenuated HVJ by genome engineering has recently been reported. Inoue et al generated



**Figure 1** Structure of Sendai virus.

**Abbreviation:** HVJ, hemagglutinating virus of Japan.

**Table 1** Various oncolytic Sendai virus

| Oncolytic Sendai virus                      | Function  |
|---|---|
| <b>Genetic modified-Sendai virus</b>        |   |
| SeV/ΔM                                      | <ul style="list-style-type: none"> <li>– Lack of production ability of infectious viral particles from infected cells</li> <li>– Infection spread by cell–cell fusion of infected cells with uninfected cells</li> </ul>  |
| MMP-sub SeV/ΔM                              | <ul style="list-style-type: none"> <li>– Preferential infection to MMP-expressing cancer cells</li> </ul>   |
| uPA-sub SeV/ΔM                              | <ul style="list-style-type: none"> <li>– Preferential infection to uPA-expressing cancer cells</li> </ul>   |
| <b>Inactivated-Sendai virus</b>             |   |
| UV-irradiated Sendai virus particle (HVJ-E) | <ul style="list-style-type: none"> <li>– Induction of apoptosis in cancer cells by introduction of viral RNA genome fragments into cytoplasm</li> <li>– Induction of necroptosis in cancer cell lack caspase-8 by HVJ-E membrane fusion</li> <li>– Activation of antitumor immunity (DCs stimulation, NK cells activation, Treg suppression)</li> </ul> |
| ΔHN-HVJ-E                                   | <ul style="list-style-type: none"> <li>– Low hemagglutinating activity</li> </ul>   |
| Tf/F-ΔHN-HVJ-E                              | <ul style="list-style-type: none"> <li>– Transferrin receptor expressing cancer cell targeting</li> </ul>   |
| sclL12-HVJ-E                                | <ul style="list-style-type: none"> <li>– Robust anticancer immune stimulation</li> </ul>  |

**Abbreviations:** ΔHN, hemagglutinin–neuraminidase-depleted; DCs, dendritic cells; HVJ-E, ultraviolet-irradiated hemagglutinating virus of Japan; MMP, matrix metalloproteinase; NK, natural killer; sclL12, single-chain interleukin-12; SeV/ΔM, recombinant hemagglutinating virus of Japan containing a viral genome lacking the M-coding region; sub, subtype; Tf/F, transferrin recombinant fusion protein; Treg, regulatory T-cell; uPA, urokinase-type plasminogen activator; UV, ultraviolet.

recombinant HVJ containing a viral genome lacking the M-coding region (SeV/ $\Delta$ M).<sup>18</sup> SeV/ $\Delta$ M exhibits the same infectivity as wild-type (wt) HVJ, but the production of mature viral particles is abolished in SeV/ $\Delta$ M-infected cells. The SeV/ $\Delta$ M infection spreads to neighboring cells by cell–cell fusion under trypsin treatment because the infected cells express F and HN. However, there is no protease for the activation of F in *Homo sapiens*, and thus the spread of SeV/ $\Delta$ M across cancer tissues by reinfection is not expected in humans. To provide the SeV/ $\Delta$ M infection with re-infectivity and cancer cell specificity, Kinoh et al generated a new SeV/ $\Delta$ M containing a gene-encoding recombinant F that is cleaved by matrix metalloproteinases (MMPs) (MMP-sub SeV/ $\Delta$ M).<sup>16</sup> It has been shown that MMP expression and activity is increased in various types of human cancers.<sup>19</sup> MMP-sub SeV/ $\Delta$ M demonstrated significantly improved infectivity and decreased tumor volume compared with both in vitro and in vivo experiment with MMP-expressing cancer cells (HT1080). However, there are many cancers which lack MMP expression, and MMP-sub SeV/ $\Delta$ M could not infect to such cancers. Kinoh et al generated another type of SeV/ $\Delta$ M that is able to undergo fusion in the presence of urokinase-type plasminogen activator (uPA), and they reported high cytotoxicity against uPA-expressing cancer cells with the uPA subtype but not MMP-sub SeV/ $\Delta$ M.<sup>17</sup>

## UV-irradiated Sendai virus particles

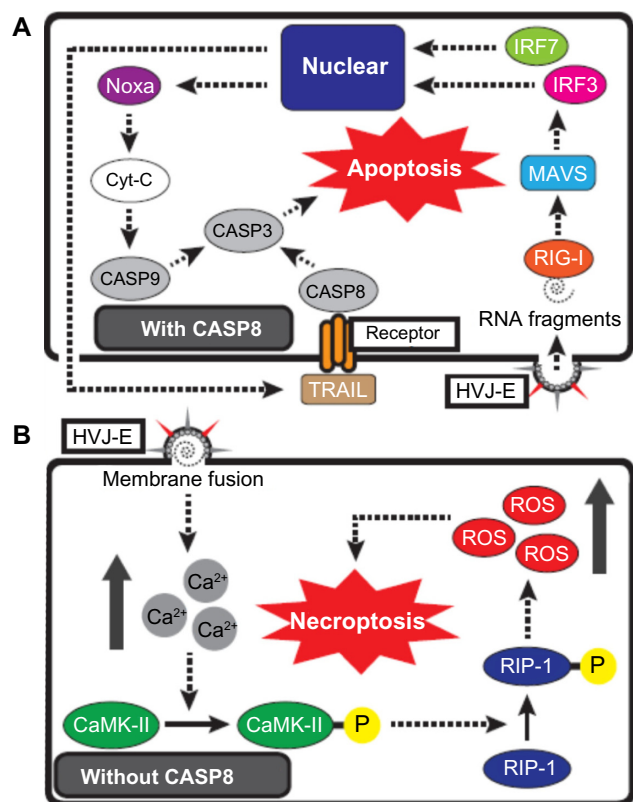
Sendai virus particles (HVJ-E) are generated via the UV irradiation of HVJ, and the viral RNA genome is fragmented into many short RNAs by UV light.<sup>20</sup> Therefore, HVJ-E maintains its membrane fusion activity but lacks the ability to replicate in infected cells. At first, HVJ-E was developed as a drug-delivery system for plasmids, siRNAs, proteins, and anticancer drugs due to its membrane fusion ability.<sup>21–24</sup> However, it was recently reported that HVJ-E directly induces cancer cell death and the activation of antitumor immunity by itself; since this discovery, research has focused on the mechanism of the antitumor effects induced by HVJ-E treatment.

## HVJ-E-induced apoptosis

Recently, many reports have shown that HVJ-E induces cancer cell apoptosis even though it is unable to replicate.<sup>25–29</sup> Kawaguchi et al demonstrated that HVJ-E effectively induces apoptosis of castration-resistant human prostate cancer cell lines (PC3 and DU145) that express high levels of GD1a and SPG (HN receptors) on the surface but does not induce apoptosis of hormone-insensitive prostate cancer cell lines

(LNCaP) that lack HN receptors.<sup>26</sup> Therefore, it was suggested that HVJ-E-mediated cell apoptosis is dependent on the expression of HN receptors. However, despite the expression of GD1a and SPG, normal prostate epithelium (PNT2) does not undergo apoptosis following HVJ-E treatment. These results suggest that HVJ-E specifically induces apoptosis in cancer cells but not normal cells; however, the mechanism of this selectivity is unclear.

Recently, the molecular mechanism of HVJ-E-mediated cancer cell apoptosis was elucidated. Tanaka et al reported that sterile alpha motif-containing domain 9 (SAMD9) is responsible for HVJ-E-induced apoptosis in human glioblastoma cells (U251MG).<sup>25</sup> They showed that HVJ-E treatment increases SAMD9 expression via type-I IFN and that SAMD9 upregulation induces U251 cell death. Moreover, Matsushima-Miyagi et al have reported more detailed mechanisms of HVJ-E-induced apoptosis in prostate cancer cells (Figure 2A).<sup>27</sup> They demonstrated that HVJ-E stimulates retinoic acid-inducible gene I (RIG-I)/mitochondrial antiviral signaling protein signaling in PC3 and DU145, and this signaling enhances the expression levels of phorbol-12-myristate-13-acetate-induced protein 1 (Noxa) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) via IRF3 and IRF 7. Noxa and TRAIL are known apoptosis inducers,<sup>30,31</sup> and these factors induce apoptosis in HVJ-E-treated cancer cells. HVJ-E has many viral RNA fragments derived from the UV-irradiated viral genome, and the RNA fragments are introduced into the cancer cell cytoplasm by HVJ-E membrane fusion. The RNA fragments are recognized by RIG-I in cancer cells, and this recognition triggers the signaling that leads to the induction of apoptosis. Therefore, it has been suggested that RIG-I is a key factor for HVJ-E-mediated cancer cell apoptosis. In fact, HVJ-E-treated cancer cells have elevated levels of RIG-I,<sup>26,27,32</sup> and RIG-I knockdown inhibits HVJ-E-mediated cancer cell apoptosis.<sup>27</sup> However, HVJ-E-mediated apoptosis is not detected in normal cells because proapoptotic genes are not induced, although the RIG-I expression level of normal cells is higher than in cancer cells under both basal and stimulated conditions in various tissue-derived cells.<sup>33</sup> Besch et al also reported that synthetic RNA induces cancer-cell-selective apoptosis via the RIG-I pathway.<sup>34</sup> In their report, apoptotic genes such as PUMA and Noxa were activated by synthetic RNA in both melanoma cells and nonmalignant skin cells, but the antiapoptotic gene BCL-xL was upregulated by the RNA only in nonmalignant cells. Thus, the mechanism of RNA-induced apoptosis in cancer cells is controversial and remains to be further elucidated. Moreover, it remains unknown why HVJ-E specifically induces the expression of proapoptotic



**Figure 2** Schematic representation of HVJ-E-mediated cancer cell death.

**Notes:** (A) HVJ-E-mediated apoptosis signaling in cancer cells with CASP8. RNA fragments, which are introduced by HVJ-E membrane fusion, are recognized by RIG-I, and the signaling induces the transcription of TRAIL and Noxa by the activation of IRF7 and IRF3 via the RIG-I/MAVS pathway. TRAIL activates CASP8 via TRAIL receptor binding, and Noxa activates CASP9 via Cyt-C release from mitochondria. Activation of CASP8 and CASP9 results in cancer cell apoptosis via the subsequent activation of CASP3. (B) HVJ-E-mediated necroptosis signaling in cancer cells without CASP8. HVJ-E membrane fusion elevates the cytoplasmic  $Ca^{2+}$  level, and  $Ca^{2+}$  induces the phosphorylation of CaMK II. CaMK-II-P phosphorylates RIP1, and RIP1-P induces cancer cell necroptosis by elevating the levels of intracellular ROS. **Abbreviations:** CaMK-II-P, phosphorylated CaMK II; CASP, caspase; Cyt-C, cytochrome-C; HVJ-E, ultraviolet-irradiated hemagglutinating virus of Japan; RIP1-P, phosphorylated RIP1; ROS, reactive oxygen species; TRAIL, TNF-related apoptosis-inducing ligand.

genes in cancer cells but not in noncancerous cells, and the elucidation of this mechanism will facilitate the understanding of the difference between cancer cells and normal cells.

## HVJ-E-induced necroptosis

As described earlier, various HVJ-E-treated cancer cells undergo apoptosis following the activation of apoptosis-related caspases (caspase-8, -9, and -3) by the recognition of RNA fragments. However, HVJ-E was recently reported to induce an entirely different cell death system from the conventional HVJ-E-mediated apoptosis.

Nomura et al reported that HVJ-E induced cell death by a mechanism other than apoptosis in human neuroblastoma cells (SK-N-SH and SK-N-AS) (Figure 2B).<sup>35</sup> First, they revealed that HVJ-E-treated SK-N-SH cells underwent cell death that was not blocked by treatment with an apoptosis

inhibitor (a pan-caspase inhibitor). SK-N-SH cells are unable to recognize HVJ-E RNA fragments because they lack RIG-I expression. Thus, the authors next focused their attention on the elevation of cytoplasmic  $Ca^{2+}$  levels following HVJ-E membrane fusion.<sup>36</sup> The  $Ca^{2+}$  increase induced the phosphorylation of RIP1 via CaMK II activation, and cell death was subsequently induced by reactive oxygen species production. Moreover, HVJ-E-mediated SK-N-SH cell death was inhibited by necrostatin-1, which is a necroptosis inhibitor. Necroptosis is a cell death process that occurs during caspase-8 inactivation, and the phosphorylation of RIP1 and RIP3 is involved in this system.<sup>37</sup> SK-N-SH does not express caspase-8, suggesting that HVJ-E can induce necroptosis in cancer cells lacking caspase-8 expression. Caspase-8 is a key factor for apoptosis, and many anticancer drugs induce cancer cell apoptosis by activating caspase-8.<sup>38</sup> However, some cancer cells acquire drug resistance through the absence of caspase-8 expression.<sup>39</sup> Therefore, it is possible that HVJ-E will be a promising treatment tool against drug-resistant cancer cells without caspase-8 expression.

## HVJ-E-mediated activation of anticancer immunity

HVJ-E induces not only cancer cell apoptosis and necroptosis but also activates anticancer immunity. Kurooka and Kaneda reported that the intratumoral administration of HVJ-E dramatically decreased the tumor volume of murine colon carcinoma (CT26) growing in the backs of mice.<sup>40</sup> Moreover, HVJ-E treatment of CT26 in the right back flank of mice significantly inhibited the engraftment of transplanted CT26 into the left back flank, suggesting that the intratumoral administration of HVJ-E activates a tumor-specific antitumor immunity. The researchers demonstrated that HVJ-E promotes the maturation of dendritic cells (DCs) and induces the production of various cytokines, namely IFN- $\alpha$  and - $\beta$ , TNF- $\alpha$  and interleukin (IL)-6, by mature DCs. In particular, IL-6 was an important factor for the activation of HVJ-E-mediated antitumor immunity, and IL-6 enhanced the proliferation and activation of cytotoxic T-lymphocytes (CTLs) by suppressing the activity of regulatory T-cells (Tregs).<sup>41</sup> When an RNA virus is detected by DCs, the viral RNA genome is recognized by Toll-like receptor (TLR)-7 and -8 in the endosome<sup>42-44</sup> or RIG-I in the cytoplasm.<sup>45</sup> However, Suzuki et al showed that IL-6 secretion by DCs is activated by reconstituted particles that contain HVJ-E components but lack an RNA genome,<sup>46</sup> suggesting that HVJ-E-mediated stimulation of DCs is independent from the recognition of RNA fragments by TLR-7, TLR-8, and

RIG-I. Moreover, they revealed that F is responsible for the induction of IL-6 secretion from DCs. However, the DC receptor that recognizes F is unknown.

It has been reported that HVJ-E induces the activation of not only CTLs but also natural killer (NK) cells. Fujihara et al reported that HVJ-E elicits antitumor activity against murine renal cell carcinoma (Renca) via NK cell activation.<sup>47</sup> They demonstrated that intratumoral HVJ-E administration induces C-X-C motif chemokine (CXCL)-10 secretion and inhibits Renca tumor growth in the backs of the mice. The HVJ-E-mediated antitumor effect against Renca was also revealed in SCID mice lacking T- and B-cells, and the effect was impaired by inhibiting NK cells with an NK cell-neutralizing antibody. CXCL10 is a chemokine for monocytes/macrophages, DCs, T-cells, and NK cells,<sup>48</sup> suggesting that HVJ-E treatment enhances the recruitment of activated NK cells by CXCL-10. HVJ-E-treated DCs, but not Renca, produce CXCL-10, and HVJ-E treatment of DCs and Renca co cultures synergistically enhance CXCL-10 secretion. Although the mechanism of the synergistic effect of CXCL-10 secretion is unknown, it is possible that HVJ-E treatment specifically induces the accumulation of active NK cells in tumor tissues but not in normal tissues.

## Modified HVJ-E

Recently, to enhance the antitumor effect and to decrease the side effects of HVJ-E, various modifications of HVJ-E were attempted. GD1a and SPG, which are receptors for HN, are expressed by nearly all cell types; therefore, HVJ-E does not have the specificity to attack only cancer cells. Moreover, red blood cells present high levels of these HN receptors on their surface, and HVJ-E induces hemagglutination in the blood. Therefore, HVJ-E could acquire cancer cell specificity and low-hemagglutinating activity for effective cancer treatment via systemic administration. To inhibit the nonspecificity and the hemagglutinating activity of HVJ-E, HN-depleted HVJ-E ( $\Delta$ HN-HVJ-E) was generated using siRNA against HN.<sup>49</sup> In comparison with the wt-HVJ-E,  $\Delta$ HN-HVJ-E exhibits low hemagglutinating activity and decreased cell adhesion, suggesting that  $\Delta$ HN-HVJ-E also has a decreased affinity for cancer cells. Shimbo et al increased the tumor-targeting efficiency of  $\Delta$ HN-HVJ-E by altering the virus to bind to the transferrin (Tf) receptor.<sup>50</sup> Tf is a carrier protein of ferric ion, and cancer cells express high levels of the Tf receptor for ferric ion uptake because ferric ion is required for DNA synthesis during active cell division.<sup>51</sup> The researchers constructed a recombinant F of Tf and F (Tf/F) and succeeded in the presentation of Tf/F on  $\Delta$ HN-HVJ-E (Tf/F- $\Delta$ HN-HVJ-E). After intravenous administration, Tf/F- $\Delta$ HN-HVJ-E, but not  $\Delta$ HN-HVJ-E, accumulated

in uterocervical cancer (HeLa) cells, expressing high levels of the Tf receptor. Moreover, Kawachi et al succeeded in the presentation of a single-chain antibody (scFv) on HVJ-E.<sup>52</sup> Many previous reports have described various tumor-associated antigens.<sup>53-56</sup> Therefore, the generation of HVJ-E showing effective cancer specificity by the presentation of scFv against tumor-associated antigens is anticipated.

The presentation of an immune-stimulatory factor on the surface of HVJ-E has been reported.<sup>57</sup> As described earlier, HVJ-E induces the production of various cytokines by DCs and enhances antitumor immunity via the downregulation of Tregs.<sup>40</sup> However, HVJ-E is unable to effectively induce Th1-inducing cytokines (eg, IL-12 and IFN- $\gamma$ ). IL-12 and IFN- $\gamma$  are important factors for the activation of antitumor immunity. IL-12 induces Th1 differentiation and IFN- $\gamma$  secretion, and IFN- $\gamma$  enhances Th1 differentiation.<sup>58</sup> Saga et al reported that HVJ-E dramatically enhances IL-12 activity and IFN- $\gamma$  secretion from murine splenocytes, much more than IL-12 alone, although HVJ-E cannot induce IFN- $\gamma$  secretion from these cells.<sup>57</sup> To enhance the activation effect of HVJ-E-mediated antitumor immunity, the researchers generated IL-12-presenting  $\Delta$ HN-HVJ-E (IL12-HVJ-E).<sup>57</sup> Compared with wt-HVJ-E, IL12-HVJ-E exhibited a robust ability to induce IFN- $\gamma$  secretion from splenocytes in vitro and significantly enhanced the antitumor immunity against mouse malignant melanomas (F10 melanoma) via intratumoral administration in vivo. Moreover, compared with wt-HVJ-E, IL12-HVJ-E reduced the lung metastatic foci of F10 melanoma via intravenous administration, suggesting the possibility of applying HVJ-E as a therapy against metastatic cancer.

## Conclusion

Here, we have described the oncolytic properties of Sendai virus, especially HVJ-E. The HVJ-E-mediated oncolytic mechanism differs from that of conventional oncolytic viruses. Recently, many reports have discussed cancer stem cells (CSCs), which are closely related to cancer malignancy.<sup>59-61</sup> CSCs are a rare population of cancer tissue and exhibit the phenotypes of drug resistance and cell growth dormancy.<sup>62</sup> Conventional oncolytic virus-mediated cell lysis requires a high proliferation of cancer cells for viral replication; therefore, conventional virotherapy might exhibit a poor effect against CSCs. However, the mechanism of HVJ-E-mediated cancer cell death is not related to cancer proliferation; consequently, it is expected that HVJ-E treatment will be effective for CSCs.

Systemic administration of HVJ-E is an attractive application for cancer therapy. To provide effective anticancer reaction by systemic HVJ-E administration, HVJ-E should be

accumulated in cancer tissue. However  $\Delta$ HN-HVJ-E, which has low hemagglutinating activity, specifically accumulates in lung by intravenous administration thereof.<sup>57</sup> Therefore, intravenous administration of  $\Delta$ HN-HVJ-E is likely to show effective therapeutic outcome against lung cancer and pulmonary metastasis but not against cancers of the other tissues. In order to effectively treat various cancers by systemic HVJ-E administration, a new highly-efficient HVJ-E delivery system to cancers has to be developed. Recently, systemic administration of platelets incorporating HVJ-E has demonstrated effective accumulation of HVJ-E to intradermal melanoma and induced robust anticancer therapeutic effect.<sup>63</sup> Development of a cancer-specific delivery system of HVJ-E will improve its therapeutic effect against cancers throughout a body by systemic HVJ-E administration.

HVJ-E-mediated cytotoxic mechanisms differ depending on the cancer cell type. Therefore, it is believed that there are unknown mechanisms of HVJ-E-mediated cancer cell death, and this process needs to be studied in greater detail. However, it is clear that HVJ-E is a robust anticancer agent that can induce both direct cytotoxicity and antitumor immunity. Moreover, HVJ-E-mediated oncolytic reactions do not need viral replication; there is no side effect of uncontrollable viral expansion which is feared in conventional oncolytic virus. Additionally, to enhance anticancer activity, armed-type HVJ-E can be easily constructed by incorporating therapeutic genes into the envelope.<sup>20,64–66</sup> Therefore, HVJ-E has a great potential for clinical application. Now, clinical trials of HVJ-E against melanoma and prostate cancer are ongoing in Japan. It is anticipated that HVJ-E will eventually be employed as a robust therapeutic tool against cancer.

## Disclosure

The authors report no conflicts of interest in this work.

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