Biomed Environ Sci, 2013; 26(12): 998-1002

Letter to the Editor

Inactivated Sendai Virus Induces Apoptosis in Murine Melanoma Cells by IGF-1R Down-regulation*

GAO Hui[1], XU Xiao Shuang[2], CHEN Ze Dong[1], ZHANG Quan[2,#], and XU Xiang Ming[2,3,#]

The mortality of cancer patients has considerably improved due to progress in surgery, chemotherapy and radiotherapy. However, some types of cancers, such as melanoma, remain refractory to conventional strategies. Although melanoma accounts for only 4% of all dermatological malignancies, it is responsible for 80% of mortalities from skin tumors[1]. The reported survival rate of melanoma over 5 years is not yet encouraging due to its chemo-resistance and rapid metastasis. Therefore, it is necessary to develop new drugs with potent activity and weak side-effect against melanoma.

The insulin-like growth factor-1 receptor (IGF-1R) belongs to a family of transmembrane protein tyrosine kinases, which is very important for cellular proliferation *in vivo*. IGF-1R is always over-expressed in tumor cells and plays a well-pronounced role in cancer development and progression[2]. Survival signals emanating from IGF-1R also inhibit apoptosis and contribute to another important receptor function involved in carcinogenesis. Moreover, the interaction between IGF-1R and IGF-1 also activates Akt and Wnt pathways[3]. IGF-1R is not an absolute requirement for normal growth, but rather plays an essential role in cells growth in transplanted tumors, which makes it an attractive target for cancer therapy[2]. Since down-regulation of IGF-1R can inhibit transformation and cause apoptosis in tumor cells[4], inhibition of IGF-1R signaling appears to be a promising strategy to interfere with the growth and survival of cancer cells. Several approaches to target IGF-1R signaling have been tried in cultured cells and in tumor xenografts, including inhibition of IGF-1R expression, blocking of ligand/receptor interaction and impairment of receptor activation[2].

Inactivated Sendai virus (Hemagglutinating virus of Japan envelope, HVJ-E) is a safe and efficient nonviral vector for drug delivery as they can incorporate DNA, RNA, proteins as well as drugs, and deliver them into cells both *in vitro* and *in vivo*[5]. In addition, HVJ-E has been applied to dendritic cell (DC) activation and used as an anticancer strategy. Recently, HVJ-E has been proven to have the ability to directly kill human prostate cancer cells (PC3) through Jak-Stat pathway. However, this HVJ-E-induced apoptosis was not completely blocked with either IFN receptor antibody or Jak inhibitor[6], suggesting that some signaling pathways other than Jak-Stat may exist to induce apoptosis in B16F10 cells by HVJ-E. It is for this reason that the present study is aimed to investigate the underlying mechanism of apoptosis induction by HVJ-E in murine melanoma cells (B16F10).

To evaluate the cytotoxic effect of HVJ-E on B16F10 cells, the cells were treated with HVJ-E at increasing MOI, and the results detected based on MTT assay revealed that HVJ-E led to a dose-dependent reduction in cell growth from 100 to 1 100 MOI (Figure 1A). Next, we sought to examine whether this reduced cell viability was due to apoptosis and IGF-1R expression post HVJ-E treatment. Annexin-V/PI double staining assay was performed to analyze B16F10 cells treated with HVJ-E at MOI of 0, 100, 200, and 400, respectively; meanwhile, cells treated with IGF-1R Inhibitor, Picropodophyllin (PPP) or transfected with siRNA targeting IGF-1R and mock siRNA were set as positive and negative controls. As shown in Figure 1B, B16F10 cells in response to HVJ-E also resulted in remarkable apoptosis in a dose-dependent manner. Moreover, PPP or siRNA targeting to IGF-1R, which could knock down the expression of IGF-1R, also induced obvious apoptosis in B16F10 cells. However, the relationship between IGF-1R and HVJ-E remains to be explained. Therefore, to further elucidate whether HVJ-E induced apoptosis was associated with IGF-1R and downstream signaling pathway, the phosphorylation of IGF-1Rβ and Akt and the expression of MITF were detected by immunoblot assay. As shown in Figure 1C, the expression of MITF

doi: 10.3967/ bes2013.037

1. College of Clinical Medicine of Yangzhou University, Yangzhou 225001, Jiangsu, China; 2. Comparative medicine Center, Yangzhou University, Yangzhou 225009, Jiangsu, China; 3. Suzhou polytechnic institute of agriculture, Suzhou 215008, Jiangsu, China

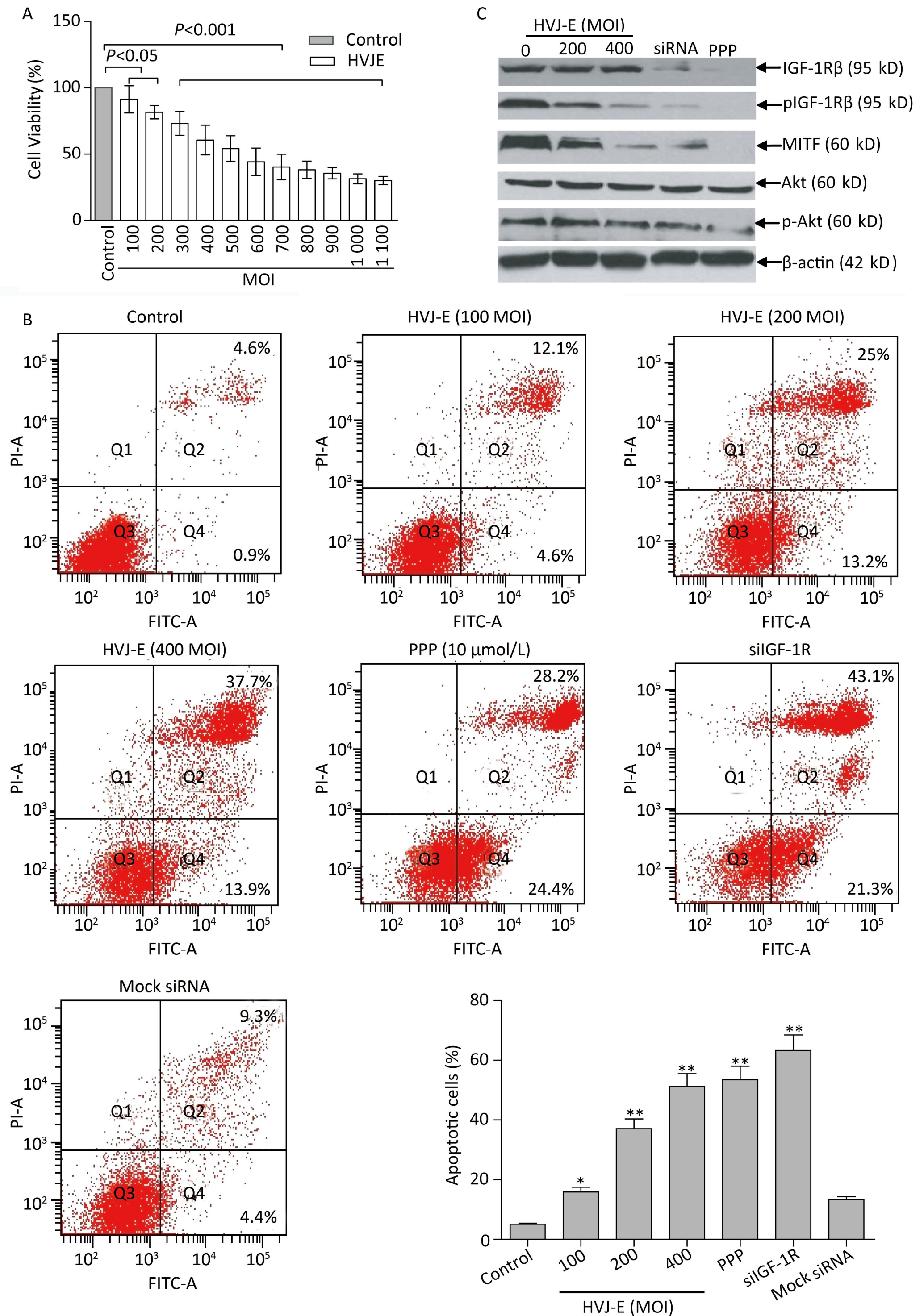

Figure 1. Apoptotic induction in B16F10 cells post HVJ-E treatment. (A) The effect of increasing MOI of HVJ-E on B16F10 cells viability detected by MTT-based assay, the data show the mean±SD of 3 experiments in quadruplicate. (B) Flow cytometric analysis of B16F10 cells treated with HVJ-E at MOI of 0, 100, 200, 400, and PPP or transfected with siRNAs for 48 h. Results are presented as mean±SD, n=3, $^{*}P$<0.05, $^{**}P$<0.01 compared with control cells. (C) B16F10 cells were treated with HVJ-E at indicated MOI, PPP, and siRNA, then the expression of MITF and the activation of IGF-1Rβ and Akt were examined with specific antibodies, total protein levels were assessed as a loading control, all experiments were performed twice.

and the activation of IGF-1Rβ and Akt were down-regulated in HVJ-E treated B16F10 cells. In accordance with other studies, our previous study also observed that apoptosis could not be completely inhibited by either Jak inhibitor or IFN α/β receptor antibody in B16F10 cells (data not shown). Therefore, these data together strongly suggest that this apoptotic induction in B16F10 cells by HVJ-E treatment may be involved in IGF-1R down-regulation, which can inhibit transformation and cause apoptosis in many types of tumor cell[4].

MITF is a master regulator gene of melanocyte development and is also associated with melanoma development. In the current experiment, the expression of MITF was decreased in B16F10 cells post HVJ-E treatment. Therefore, to evaluate the ability of HVJ-E in melanogenesis inhibition, the tyrosinase activity was assayed by quantifying DOPA oxidation, since tyrosinase is known to be a rate-limiting enzyme in melanin synthesis. The obtained results showed that in B16F10 cells, tyrosinase activity was significantly down-regulated (P<0.05) by HVJ-E treatment in a dose-dependent manner (Figure 2A). To exclude a direct influence of HVJ-E on tyrosinase activity, we performed an *in vitro* study by directly adding HVJ-E to B16F10 control cell lysates and then measuring tyrosinase activity, and our findings demonstrated that HVJ-E had no direct effect on tyrosinase activity (Figure 2B). As shown in Figure 2C, the melanin contents measured in HVJ-E-treated cells was also significantly decreased (P<0.05). Collectively, these findings suggest that HVJ-E could inhibit the expression of MITF, which resulted in the reduction of melanin synthesis in HVJ-E-treated B16F10 cells.

To further evaluate the anti-tumor effect of HVJ-E *in vivo*, BALB/c nude mice (6-week old) were divided into two groups, with each group containing 10 mice. B16F10 cells (2×10^6) were intradermally injected into the backs of BALB/c nude mice. After tumors inoculated into the mice reached 5 mm in diameter, HVJ-E (1×10^9) or PBS was injected into tumors. Three days later, the tumors of 3 mice in each group were removed and tumor sections were examined by immunohistochemistry (IHC) and TUNEL assay. IHC assay of the HVJ-E-treated tumor sections showed that the expression of IGF-1Rβ was remarkably inhibited, while IGF-1R was highly displayed in PBS treated tumors (Figure 3A). In addition, the results from TUNEL assay revealed pyknotic chromatin in HVJ-E-treated tumor sections (Figure 3B). In contrast, fewer tumor necroses or apoptotic cells were observed in PBS-treated tumor sections. Furthermore, tumor volume analysis also demonstrated effective inhibition of melanoma tumor growth in HVJ-E-injected BALB/c nude mice compared with control mice (Figure 3C), indicating that down-regulation of IGF-1R by HVJ-E could directly induce apoptosis in B16F10 melanoma cells and delay tumor growth.

To date, IGF-1R inhibition has been studied in both cultured cells and tumor xenografts. However, IGF-1R down-regulation has been shown to have a very modest effect on cells in monolayer culture, while

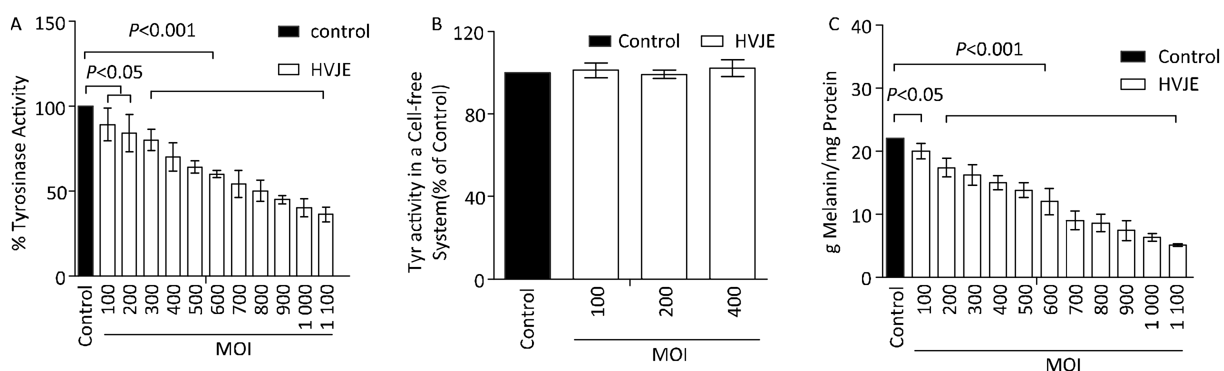

Figure 2. Tyrosinase activity and melanin production in HVJ-E-treated B16F10 cells. (A) Tyrosinase activity measured by L-DOPA oxidation using lysates obtained from B16F10 cells treated with HVJ-E for 48 h. (B) The direct effect of HVJ-E on tyrosinase activity was determined by adding HVJ-E of indicated MOI to B16F10 control cell lysates. (C) Following incubation with indicated MOI of HVJ-E for 48 h, the extracellular and intracellular melanin was determined separately. Standard curves of synthetic melanin were used to extrapolate the absolute values of melanin content. The total amount of melanin was calculated for each experimental point by adding the extracellular and intracellular melanin values after normalization for protein content. The data show the mean±SD of three experiments performed in quadruplicate.

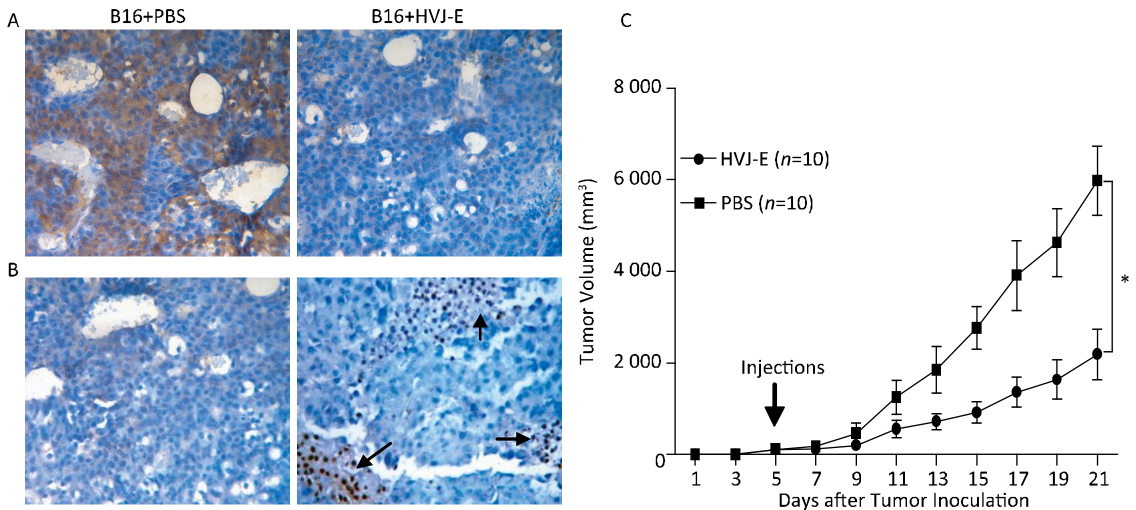

Figure 3. HVJ-E inhibits the expression of IGF-1R and induces apoptosis *in vivo*. Murine melanoma-bearing BLAB/c nude mice were either treated with PBS buffer or intratumoral injection of HVJ-E. On day 3 post HVJ-E-treatment, 3 mice of each group were sacrificed and tumors were processed for analyses. (A) Immunohistochemistry examination of IGF-1R expression in PBS- or HVJ-E-treated tumors (400×). (B) TUNEL staining of apoptotic cells in PBS- or HVJ-E-treated tumors (brown 3,30-diaminobenzidine chromogen in cell nuclei; arrow, 400×). (C) Tumor volumes were measured at 2-day intervals for 21 days after injections and expressed as the mean±SD (n=10) in tumor volume-time curves, The HVJ-E-treated tumors were effectively inhibited as compared with PBS-treated tumors. $^*P$<0.05.

cells transplanted as xenografts into mice are more drastically affected by impairment of IGF-1R signaling[7]. In contrast, the role of IGF-1R down-regulation in robust apoptosis induction in melanoma cells was also reported[8], which was consistent with our findings. Nevertheless, the controversial results indicate that the specific mechanism by IGF-1R is still not fully clear.

A few reports have shown that intratumoral injection of HVJ-E could inhibit tumor growth effectively and prolong the survival period of tumor-bearing mice, and that these potential mechanisms by HVJ-E may be involved in immune responses stimulation, particularly the T cells mediated cytotoxic T lymphocytes (CTL) effects[9-10]. In the present experiment, BALB/c nude mice, which were deprived of T cells, were used to exclude the T cell-mediated immune responses caused by HVJ-E, and as shown, the tumors were effectively inhibited post HVJ-E injection. Taken together, this result indicated that HVJ-E could inhibit the IGF-1R expression not only *in vitro*, but also *in vivo*, and the down-regulation of IGF-1R may contribute to apoptosis induction by HVJ-E.

[*]This work was supported by Natural Science Foundation of Jiangsu Province (BK2011049), Jiangsu “333”Projects in Jiangsu Province (BK201140032) and Innovation Fund of Yangzhou University (2012CXJ085).

[#]Correspondence should be addressed to Dr ZHANG Quan and XU Xiang Ming (zquan@yzu.edu.cn; yzxmxu@163.com)

Biographical note of the first author: GAO Hui, female, born in 1961, associate professor and PhD, majoring in dermatology medicine.

Received: March 18, 2013;

Accepted: May 4, 2013

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin, 2007; 57, 43-66.
2. Ackermann M, Morse BA, Delventhal V, et al. Anti-VEGFR2 and anti-IGF-1R-Adnectins inhibit Ewing's sarcoma A673-xenograft growth and normalize tumor vascular architecture. Angiogenesis, 2012; 15, 685-95.
3. Bellei B, Flori E, Izzo E, et al. GSK3beta inhibition promotes melanogenesis in mouse B16 melanoma cells and normal human melanocytes. Cellular signalling, 2008; 20, 1750-61.
4. Shu S, Yang Y, Li X, et al. Down-regulation of IGF-1R expression inhibits growth and enhances chemosensitivity of endometrial carcinoma *in vitro*. Molecular and cellular biochemistry, 2011; 353, 225-33.
5. Suvanasuthi S, Tamai K, and Kaneda Y. Rapid transport of plasmid DNA into the nucleolus via actin depolymerization using the HVJ envelope vector. J Gene Med, 2007; 9, 55-62.
6. Kaneda Y. A non-replicating oncolytic vector as a novel therapeutic tool against cancer. BMB reports, 2010; 43, 773-80.

7. Bahr C and Groner B. The insulin like growth factor-1 receptor (IGF-1R) as a drug target: novel approaches to cancer therapy. Growth Horm IGF Res, 2004; 1, 287-95.
8. Jiang YY, Huang H, Wang HJ, et al. Interruption of mitochondrial complex IV activity and cytochrome c expression activated O(2).(-)-mediated cell survival in silibinin-treated human melanoma A375-S2 cells via IGF-1R-PI3K-Akt and IGF-1R-PLC gamma-PKC pathways. European journal of pharmacology, 2011; 668, 78-87.
9. Kurooka M and Kaneda Y. Inactivated Sendai virus particles eradicate tumors by inducing immune responses through blocking regulatory T cells. Cancer research, 2007; 6, 227-36.
10. Zhang Q, Yuan WF, Zhai GQ, et al. Inactivated Sendai Virus Suppresses Murine Melanoma Growth by Inducing Host Immune Responses and Down-regulating β-catenin Expression. Biomed Environ Sci, 2012; 25, 509-16.