

腫瘍標的抗体とHVJ-Eによる新規癌標的治療剤の開発

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Development of a novel cancer therapy using tumor-targeting HVJ-E vector

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1. Hatano K, Miyamaoto Y, Nonomura N, Kaneda Y. (2011)

Expression of gangliosides, GD1a and sialyl paragloboside, is regulated by NF- κ B-dependent transcriptional control of α 2,3-sialyltransferase I, II and VI in human castration-resistant prostate cancer cells.

Int J Cancer, 2010 Dec 16. [Epub ahead of print]

Gangliosides are sialic acid-containing glycosphingolipids that are associated with tumor malignancy and progression. Among the enzymes required for the production of gangliosides, sialyltransferases have received much attention in terms of their relationship with cancer. In our previous report, ganglioside GD1a and sialyl paragloboside (SPG), a neolacto-series ganglioside, were much more abundant in PC3 and DU145 cells, castration-resistant prostate cancer cells, as compared with hormone-sensitive prostate cancer cells and normal prostate epithelium. GD1a is synthesized from GM1 by α 2,3 sialyltransferase (ST3Gal) I and mainly by ST3Gal II. The enzyme to synthesize SPG is ST3Gal VI. The high production of GD1a and SPG in castration-resistant prostate cancer cells was correlated with the high expression of ST3Gal II and VI, respectively. The expression of ST3Gal I and II was mildly induced by phorbol-12-myristate-13-acetate (PMA), and PMA-induced expression of ST3Gal I and ST3Gal II was inhibited by NF- κ B decoy oligodeoxynucleotides (ODN) but not by AP-1 decoy ODN. Among the five mammalian homologs of the NF- κ B family, RelB RNAi most effectively inhibited the expression of ST3Gal I and ST3Gal II. The expression of ST3Gal VI was also most effectively inhibited by RelB RNAi. The amount of GD1a and SPG was significantly reduced by RelB siRNA treatment in PC3 cells. Thus, the production of GD1a and SPG in castration-resistant prostate cancer cells was indirectly controlled by NF- κ B, mainly by RelB, through the transcriptional regulation of ST3Gal I, II and VI.

2. Matsuda M, Yamamoto T, Matsumura A, Kaneda Y. (2009)

Highly efficient eradication of intracranial glioblastoma using Eg5 siRNA combined with HVJ envelope.

Gene Therapy, 16: 1465-1476.

Hemagglutinating virus of Japan envelope (HVJ-E) vector with inactivated replication-defective Sendai virus was originally developed as a versatile drug delivery system. Recently, we have demonstrated direct tumor-killing activity of HVJ-E itself without therapeutic molecules in prostate cancer cells. Although human glioblastoma cells were also sensitive to HVJ-E treatment, complete eradication was not achieved using HVJ-E alone. Here, to develop more effective therapeutic strategy of glioblastoma, we enhanced the anti-tumor activity by incorporating siRNA of mitotic motor protein Eg5 into HVJ-E. Treatment with HVJ-E containing Eg5 siRNA induced monopolar spindle formation and resulted in cell-cycle arrest and apoptosis. When HVJ-E containing Eg5 siRNA was directly injected into an intradermal tumor xenograft, all mice became tumor-free. Similar results were observed in intracranial tumor xenografts. The survival time of treated mice was significantly prolonged when HVJ-E was used. Histological examination revealed that tumors remained in the brain following treatment with HVJ-E containing control siRNA, but no tumors were detected following treatment with HVJ-E containing Eg5 siRNA. This remarkable anti-tumor response was likely due to a synergistic effect of Eg5 siRNA and HVJ-E. Thus, this combination shows promise as an attractive novel therapy for glioblastoma.

3. Kawaguchi Y, Miyamoto Y, Inoue T, Kaneda Y. (2009)

Efficient eradication of hormone-resistant human prostate cancers by inactivated Sendai virus particle.

Int J Cancer, 124: 2478-2487.

Hormone-refractory prostate cancer is one of the intractable human cancers in the world. Here, we examined the direct tumor-killing activity of inactivated Sendai virus particle (hemagglutinating virus of

Japan envelope; HVJ-E) through induction of type I interferon (IFN) in the hormone-resistant human prostate cancer cell lines PC3 and DU145. Preferential binding of HVJ-E to PC3 and DU145 over hormone-sensitive prostate cancer cell and normal prostate epithelium was observed, resulting in a number of fused cells. After HVJ-E treatment, a number of IFN-related genes were up-regulated, resulting in type I IFN production in PC3 cells. Then, RIG-I (retinoic acid-inducible gene-I) helicase which activates type I IFN expression after Sendai virus infection was up-regulated in cancer cells following HVJ-E treatment. Produced IFN- α and - β enhanced caspase 8 expression via JAK-STAT pathway, activated caspase 3 and induced apoptosis in cancer cells. When HVJ-E was directly injected into a mass of PC3 tumor cells in SCID mice, a marked reduction in the bulk of each tumor mass was observed and 85% of the mice became tumor-free. Although co-injection of an anti-asialo GM1 antibody with HVJ-E into each tumor mass slightly attenuated the tumor suppressive activity of HVJ-E, significant suppression of tumor growth was observed even in the presence of anti-asialo GM1 antibody. This suggests that NK cell activation made small contribution to tumor regression following HVJ-E treatment in hormone-resistant prostate cancer model in vivo. Thus, HVJ-E effectively targets hormone-resistant prostate cancer by inducing apoptosis in tumor cells, as well as activating anti-tumor immunity.

4. Suzuki H, Kurooka M, Hiroaki Y, Fujiyoshi Y, Kaneda Y. (2008)

Sendai virus F glycoprotein induces IL-6 production in dendritic cells in a fusion-independent manner.

FEBS Letters, 582: 1325-1329.

We previously reported that inactivated Sendai virus particle (hemagglutinating virus of Japan envelope; HVJ-E) has anti-tumor effects by eliciting IL-6 production in dendritic cells (DCs). In the present study, we investigated which components of HVJ-E elicit IL-6 production. HVJ-E containing F0 protein inactive for virus envelope-cell membrane fusion enhanced IL-6 production. Reconstituted liposomes containing F protein stimulated IL-6 production. The antibody against F protein inhibited IL-6 secretion by HVJ-E. When carbohydrate chains of the F glycoprotein were removed, HVJ-E lost the ability to stimulate IL-6 secretion. These results suggest that F glycoprotein is required for IL-6 production in DCs.

5. Kurooka M, Kaneda Y. (2007)

Inactivated Sendai virus particles eradicate tumors by inducing immune responses through blocking regulatory T cells.

Cancer Res, 67: 227-236.

Ultraviolet-inactivated, replication-defective Sendai virus particles (Hemagglutinating virus of Japan envelope, HVJ-E) injected into murine colon carcinoma (CT26) tumors growing in syngeneic Balb/c mice eradicated 60-80% of the tumors and obviously inhibited the growth of the remainder. Induced adaptive anti-tumor immune responses were dominant in the tumor eradication process because the effect was abrogated in severe combined immunodeficient (SCID) mice. Murine and human dendritic cells (DCs) underwent dose-dependent maturation by HVJ-E in vitro. Profiles of cytokines secreted by DCs after HVJ-E stimulation showed that the amount of IL-6 released was comparable to that elicited by live HVJ. Real-time RT-PCR and immunohistochemistry revealed that HVJ-E induced a remarkable infiltration of DCs, CD4⁺ and CD8⁺ T cells into tumors. And CT26 specific cytotoxic T lymphocytes (CTL) were induced with the evidence of enhanced CD8⁺ T cell activation in a CD4⁺CD25⁻ T cell-dependent manner. On the other hand, conditioned medium from DCs stimulated by HVJ-E (H-DCCM) rescued CD4⁺CD25⁻ effector T cell proliferation from Foxp3⁺CD4⁺CD25⁺ regulatory T cell (Treg) mediated suppression and IL-6 was presumably dominant for this phenomenon. We also confirmed such rescue in mice treated with HVJ-E in vivo. Moreover, anti-tumor effect of HVJ-E was significantly reduced by an in vivo blockade of IL-6 signaling. This is the first report to show that HVJ-E alone can eradicate tumors and the mechanism through which it induces anti-tumor immune responses. Because it can enhance anti-tumor immunity and simultaneously remove Treg mediated suppression, HVJ-E shows promise as a novel therapeutic for cancer immunotherapy.