# 論　文　内　容　の　要　旨
# Synopsis of Thesis

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| 論文題名 Title | Virus-stimulated neutrophils in the tumor microenvironment enhance T cell-mediated anti-tumor immunity (腫瘍微小環境内におけるウイルス刺激好中球によるT細胞活性化を介した抗腫瘍免疫効果) |

論文内容の要旨

〔目　的(Purpose)〕

Major conventional cancer therapies include surgery, chemotherapy and radiation therapy. These therapies have been useful and advances in medical science have aid progress in cancer therapy, but there are still limitations with regards to cancer recurrence and metastases targeting. To resolve these problems, anti-tumor immunotherapy has been an attractive target recently. Inactivated Sendai virus particles (hemagglutinating virus of Japan envelope; HVJ-E) have been demonstrated to induce activation of anti-tumor immunity by elevating immune activation and suppressing regulatory T cells. Even though HVJ-E itself is an effective agent to stimulate anti-tumor immunity, it may also have potential to be used in combination treatment to increase anti-tumor effect. HVJ-E activates anti-tumor immunity mainly through RIG-I/MAVS signaling pathway, and is independent of Toll-like receptor (TLR) pathways. In this research, we attempted to examine whether a RIG-I agonist and TLR agonist can synergistically activate anti-tumor activity. To investigate this aim, we injected HVJ-E with or without poly I:C (TLR3 agonist) into B16-F10 mouse melanoma tumor model.

〔方法ならびに成績(Methods/Results)〕

Tumor growth was significantly suppressed by combination treatment of HVJ-E and poly I:C. We found that poly I:C treatment recruited abundant neutrophils to tumor tissue. To investigate the mechanism, we used cytokine array and our data showed neutrophil chemotactic CXCL2 produced from the tumor bed by poly I:C, but not HVJ-E. Tumor associated neutrophils (TANs) are traditionally considered to favor tumor development, but N1 phenotype of TANs can suppress tumor growth. We used FACS analysis to investigate the accumulation of neutrophils and their markers. We found that HVJ-E directly or indirectly up-regulated FAS and ICAM-1 expression on neutrophils, which resulted in the polarization of TANs to N1 type. Both neutrophil depletion and CXCL2 neutralization decreased synergistic anti-tumor effect of HVJ-E and poly I:C combination treatment. Our results indicated that poly I:C and HVJ-E can activate anti-tumor effect via the conversion of recruited neutrophils to N1 phenotype. Because of high toxicity of poly I:C treatment, we developed gene therapy using HVJ-E containing CXCL2 gene. Intratumoral injection of CXCL2 gene-loaded HVJ-E suppressed mouse melanoma in vivo by increasing cytotoxic T lymphocytes against melanoma.

〔総　括(Conclusion)〕

In all, HVJ-E combined with poly I:C or neutrophil chemotactic CXCL2 increased anti-tumor effect by inducing neutrophil recruitment and conversion of TANs to N1 type. N1 polarization is a new function of HVJ-E-mediated anti-tumor activities and CXCL2 gene-loaded HVJ-E may provide a new tool for cancer immunotherapy.

# 論文審査の結果の要旨及び担当者

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## 論文審査の結果の要旨

本研究は不活性化センダイウイルス粒子(HVJ-E)とToll-like receptor (TLR) agonistの併用投与による新規癌治療法の開発である。メラノーマのモデルマウスにHVJ-EとTLR-3 agonist(poly I:C)の併用投与を行った結果、HVJ-Eの単独投与より強い相乗的な抗腫瘍効果が認められた。その作用機序は、まず、poly I:Cの投与によって好中球遊走因子CXCL2が産生され、好中球が腫瘍組織に集積する。そこで、HVJ-Eが好中球に作用し、抗腫瘍活性を持つN1 typeに変化させることがわかった。腫瘍内で抗腫瘍活性を持つ好中球が増加することにより、細胞傷害性T細胞(CTL)活性が増強し、腫瘍抑制を引き起こした。この機構を利用し、HVJ-Eと好中球遊走因子CXCL2タンパクを併用投与、及び、CXCL2遺伝子を封入したHVJ-Eを用いた遺伝子治療によって、好中球を介したCTL活性化による抗腫瘍効果が見られた。また、メラノーマのモデル以外に、乳腺がん（4T1）と大腸がん（MC38）のマウスモデルでも好中球を介した抗腫瘍効果が得られた。In vitroの実験において、HVJ-Eは直接的、間接的に作用して、好中球をN1 typeに変化させ、そのN1 typeの好中球とT細胞を共培養した結果、CTL活性が増強された。

以上より、本研究は、HVJ-Eの好中球に対する新たな作用を発見し、それをもとにした新規癌免疫治療法を実現したことから学位の授与に値すると考えられる。