論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	Li-Wen Liu
論文題名 Title	An RNA Molecule Derived from Sendai Virus DI Particles Induces Antitumor Immunity and Cancer Cell-selective Apoptosis (センダイウイルス由来DI粒子による抗腫瘍免疫および癌細胞選択的アポトーシスの誘導)

論文内容の要旨

〔目 的(Purpose)〕 We have already reported that a non-replicating Sendai virus (hemagglutinating virus of Japan) envelope (HVJ-E) stimulates anti-cancer immunity and cancer cell-selective apoptosis. In cancer cells, HVJ-E RNA genome fragments are recognized by the cytoplasmic RNA receptor retinoic acid-inducible gene-I (RIG-I) and trigger mitochondrial antiviral signaling (MAVS) protein to activate interferon regulatory factor (IRF) 3 and 7. Through this signaling pathway, cancer cell-selective apoptosis was induced by activating proapoptotic genes, TNF-related apoptosis-inducing ligand (TRAIL) and Noxa. The virus RNA pathogen-associated molecular patterns (PAMPs) characteristics of oncolytic virotherapy are not well understood. In a previous study, the Cantell strain HVJ promoted greater DC maturation and type I IFN production than the Z strain HVJ. It has been proven that viral defective-interfering (DI) particles in the Cantell strain of HVJ contribute to the Toll-like receptor (TLR)-independent immunostimulatory ability. Cantell DI particles contain incomplete viral genomes which have complementary termini forms the stem region of the frying-pan-shaped secondary RNA structure and was reported to exhibit the highest binding affinity to RIG-I. In this study, we focused on DI particle of Cantell strain HVJ and analyzed the virus RNA PAMPs characteristics which are required for the cancer cell-selective anti-cancer effects.

〔方法ならびに成績(Methods/Results)〕 We first showed that β-propiolactone-treated Cantell strain HVJ induced the RIG-I/MAVS signaling pathway and proapoptotic proteins expression in cancer cells. Moreover, the same amount of gel-extracted DI RNA induced a higher level of expression of those apoptosis-related proteins and more cell death than the whole-genome RNA did. Thus, the anti-cancer effect of DI RNA genome was confirmed. Next, we examined whether a specific secondary structure of the DI RNA genome stimulates the RIG-I/MAVS downstream-related cancer suppressive pathways using HVJ-derived in vitro transcription (IVT) RNAs. IVT-B2 which was derived from Cantell HVJ DI genome had a secondary structure with a dsRNA terminus and an ssRNA loop, strongly stimulated RIG-I dependent proapoptotic proteins induction in prostate cancer cells. In contrast, the IVT-HN which was from part of the Z strain HVJ HN gene which has no specific secondary structure, had no impact on inducing anti-cancer effects. We also showed that when IVT-B2 was modified by deleting 3' or 5' portions of the ssRNA region did not affect on the cancer cell-selective killing effect. However, the IVT-DI RNA with shorter dsRNA regions had no activity of cancer cell killing and hardly upregulated the expression of proapoptotic genes. We also found that calf intestinal alkaline phosphatase-treated IVT-B2 RNA lost the capability of inducing RIG-I/MAVS-related downstream Noxa and TRAIL expression. Finally, transfection of IVT-B2 to xenograft prostate tumor induced intratumoral TRAIL expression and apoptosis, and resulted in extensive reducing of tumor volume *in vivo*.

〔総 括(Conclusion)〕

DI particle-derived IVT-B2 RNA, which has a double-stranded region in its secondary structure, promoted a stronger RIG-I dependent anti-cancer effect than IVT-HN RNA, which does not have a double-stranded region in its secondary structure. Both the double-stranded stem and 5'-triphosphate of IVT-B2 RNA are crucial in inducing the cancer cell-selective apoptosis. In this study, the virus RNA PAMPs characteristics of those in cancer treatment were clarified through investigating the DI particle RNA genome with respect to its anti-cancer effect. These findings provide a novel nucleic acid medicine for the treatment of cancer.

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

不活性化センダイウイルス粒子（hemagglutinating virus of Japan envelope; HVJ-E）には抗腫瘍免疫および癌細胞選択的アポトーシスを惹起することが既に報告されている。癌細胞においてHVJ-EのRNAゲノム断片は細胞質内RNA受容体であるRIG-I（retinoic acid-inducible gene-I）によって認識され、さらにMAVS（mitochondrial antiviral signaling）によってシグナル下流に存在するTRAIL（TNF-related apoptosis-inducing ligand）やNoxaなどのアポトーシス促進性遺伝子の活性化やIFN-βなどのサイトカインやケモカインの誘導を促す。本研究では、HVJカンテル株に豊富に存在するDI(defective interfering)粒子由来のRNAゲノムを解析することにより、RIG-Iによって認識されその下流のシグナル伝達に必要なウイルスRNAの構造解明を行った。DI粒子のRNAゲノム配列をもとに人工的に合成されたIVT-B2 RNAはその一部に二重鎖構造を持ち、この特徴的な構造が強力にRIG-I依存的な抗腫瘍効果を引き起こすことが確認された。加えて、IVT-B2がもつ5’末端の三リン酸部位もまたアポトーシス促進性遺伝子やIFN-βの誘導に重要な因子であることが見出された。さらに、ヒト前立腺癌細胞移植マウスモデルに対してIVT-B2 RNAの直接投与を行ったところ、顕著な腫瘍サイズの縮小がみられ、投与後の腫瘍内におけるアポトーシス細胞の増加、NK細胞による抗腫瘍効果の増強が観察された。

これらの発見は癌治療における新規核酸薬の開発に繋がる業績であり、博士(医学)の学位授与に値する。