主論文の要旨

Combination of Cetuximab and Oncolytic Virus Canerpaturev Synergistically Inhibits Human Colorectal Cancer Growth

（セツキシマブと腫瘍溶解性ウイルスカネパチューレブの併用療法は相乗作用的にヒト結腸直腸癌の増殖を阻害する）

名古屋大学大学院医学系研究科　総合医学専攻
病態外科学講座　消化器外科学分野
（指導：小池 聖彦　特命教授）
吴 智闻

【INTRODUCTION】

Colorectal cancer (CRC) is the third most common cancer worldwide. The morbidity and incidence of this cancer are expected to be increased in the coming decades. Accordingly, there is a desperate need for multimodal therapy to improve outcomes in patients with CRC.

Oncolytic viruses have been proposed as a promising and high effective therapy for cancer treatment. Canerpaturev (C-REV) is a spontaneous mutant clone derived from HF, a highly attenuated mutant strain of HSV-1, which showed promise in our previous studies. Epidermal growth factor receptor (EGFR) signaling is involved in apoptosis, angiogenesis, cell proliferation, migration, and invasion. Salomo et al. reported EGFR overexpression (72–82% above normal tissue) in colorectal cancer. Cetuximab, a monoclonal antibody that binds to the extracellular domain of EGFR, has been applied widely to suppression of tumor growth. In this study, we combined C-REV and cetuximab to treat human colorectal cancer and evaluated the anti-tumor efficacy of this regimen. Cetuximab treatment prior to C-REV treatment strongly inhibited tumor growth by enhancing virus spread and preventing angiogenesis.

【MATERIALS AND METHODS】

The human colorectal cancer cell line HT-29, WiDr and CW2 were used in this experiment. C-REV is a highly attenuated mutant clone derived from HSV-1 strain HF. The virus was propagated in Vero cells and stored in aliquots at −80°C.

EGFR expression was measured in a variety of human colorectal cancer cell lines by western blot and flow cytometry. We performed MTT assay to evaluate the cell proliferation in vitro. We evaluate the effect of Cetuximab on viral replication by performing virus titering. Furthermore, in vivo study, we investigate the tumor growth inhibition by giving both C-REV and Cetuximab, which was compared with other three groups. Immunohistochemistry staining was performed to detect HSV-1 and CD31 in tumor samples.

【RESULTS】

EGFR Expression Level in Colorectal Cancer Cell Lines

HT-29 expressed the highest level of EGFR, and CW2 the lowest (Figure 1A, 1B). EGFR expression of all three cell lines was reduced 3 days after C-REV (MOI 1) infection (Figure 1C, Figure S1). This result suggested that C-REV infection directly modulates EGFR expression in colorectal cancer cell lines.

Cytotoxicity of Cetuximab and C-REV in Colorectal Cancer Cell Lines and Cetuximab Has No Effect on Viral Replication

C-REV exerted a strong cytotoxic effect on all three cell lines, and its effect was time- and dose-dependent. Cetuximab alone had a slightly cytotoxic effect *in vitro* (Figure 2A), and combination therapy with cetuximab and C-REV had no additive effect (Figure 2B). Cetuximab had no effect on viral replication in any of the three cell lines (Figure 2C).

Combination Therapy with Cetuximab and C-REV Exerts Strong Anti-tumor Effect in HT-29 Tumor Xenografts

We applied two kinds of treatment regimens to our tumor model (Figure 3A and 3D) and compared their efficacy. Combination G1 suppressed tumor growth significantly relative to either single therapy (Figure 3B); Combination G2 was superior to the control and cetuximab group but was not significantly differ from the C-REV group (Figure 4E). Based on measurement of fractional tumor volume (FTV), Combination G1 synergistically inhibited tumor growth (Tables 1). No adverse effects were observed in the tumor model, as assessed by evaluation of body weight (Figure 3C).

Antiangiogenic Effect is Enhanced Significantly in Combination Therapy

HSV-1 virus distribution within the tumor was enhanced in the Combination G1 group relative to the C-REV group on Day 3 (Figure 4A and 4B). Cetuximab enhanced the antiangiogenic effect on Day 14 in the Combination G1 group relative to the C-REV group (Figure 4C). Meanwhile, both CD31 staining and microvessel density (MVD) revealed that the antiangiogenic effect was significantly enhanced in Combination G1 relative to Combination G2 (Figure 4C and 4D). The antiangiogenic effect was consistent with the *in vivo* antitumor effect of combination therapy.

【DISCUSSION】

Preclinical studies and clinical trials over the past decades have shown that oncolytic viruses have potential efficacy against various tumors, but the inefficient distribution capability of virus in tumors always limited clinical efficacy. Oncolytic virus distribution is highly affected by the tumor microenvironment. High levels of secretion of extracellular matrix proteins by tumor cells contribute to high interstitial fluid pressure (IFP), which is one of the major factors preventing viruses from spreading in tumors. Because IFP increases as the tumor grows, the tumor angiogenesis is closely linked to high IFP. Therefore, antiangiogenic therapy has been proposed as a means to promote more efficient viral distribution.

Inhibition of EGFR by tyrosine kinase inhibitors or monoclonal antibodies decreases the angiogenic profile of tumor cells, whereas overexpression of EGFR on tumor cells is related to the production of angiogenic molecules. These findings indicated that cetuximab has an antiangiogenic effect. In this study, immunohistochemical staining for CD31

revealed that the combination of cetuximab and C-REV decreased tumor angiogenesis relative to C-REV treatment alone (Figure 4C and 4D). HSV can induce angiogenesis in various tumors, and viral infection induces production of VEGF. Hence, inhibition of angiogenesis by cetuximab improved the antitumor effect of C-REV in the HT-29 xenograft model by promoting efficient virus distribution within the tumor.

In addition, the decrease in EGFR expression after treatment with C-REV is related to the therapeutic effects of cetuximab. Liang et al. demonstrated that HSV-1 ICP0 can interact with CIN85 and Cbl, forming a complex that downregulates cell surface levels of EGFR in the absence of EGF. Their result is in accordance with our finding that expression of EGFR on three colorectal cancer cell lines decreased following C-REV infection (Figure 1C, Figure S1). This result may explain that C-REV prior to cetuximab combination therapy had no additive inhibitory effect relative to the C-REV group. Moreover, C-REV–induced angiogenesis was not inhibited by cetuximab *in vivo* (Figure 4C, Combination G2). However, other researchers reported that the interaction between EGFR and PI3K was up-regulated, and EGFR was transiently activated, during HSV-1 infection. We also determined that expression of EGFR *in vitro* was temporarily increased after C-REV administration, but subsequently decreased (Figure S2). Further studies are needed to explore the internal mechanism underlying this finding.

【CONCLUSION】

Our results show that the combination of the anti-EGFR monoclonal antibody cetuximab and the oncolytic virus C-REV induced a synergistic antitumor effect in human colorectal cancer xenograft model. Cetuximab enhanced the anti-tumor activity of C-REV by promoting viral distribution and inhibiting angiogenesis. Our findings suggest that applying cetuximab prior to C-REV can gain more benefit in tumor growth inhibition. With further investigation, combination therapy could be developed into an effective antitumor strategy against human colorectal cancer.