主論文の要旨

# Combination therapy of oncolytic herpes simplex virus HF10 and Bevacizumab against experimental model of human breast carcinoma xenograft

（ヒト乳癌マウスモデルに対するヘルペスウイルスHF10とベバシズマブの相乗効果についての研究）

名古屋大学大学院医学系研究科　機能構築医学専攻
病態外科学講座　消化器外科学分野
（指導：小寺 泰弘　教授）
谭 戈文

**BACKGROUND**

Breast cancer is one of the most common and feared cancers faced by women. According to the latest statistics on cancer, breast cancer has already been the second leading cause of death for women in the United States. However, the treatment of patients who are diagnosed at an advanced stage and curative surgical treatments are sometimes difficult due to the presence of recurrence and metastases. Furthermore, the long-term prognosis of curatively resected advanced breast cancer remains unsatisfactory because of its high recurrence rate after surgery. Currently the available chemotherapeutic reagents have only limited efficacy against these recurrent diseases. In particular, the prognosis of patients with advanced or recurrent breast cancer remains poor despite refinements in multimodality therapies involving chemotherapeutic and hormonal agents. Multimodal therapy with a more specific and effective strategy is urgently needed. Oncolytic HSV has potential to become a new effective treatment option because of its broad host range and tumor selective viral distribution. Bevacizumab is a monoclonal antibody against VEGFA which inhibits angiogenesis and therefore tumor growth. Our approach to enhance the antitumor effect of the oncolytic HSV is to combine oncolytic HSV HF10 and Bevacizumab in the treatment of breast cancer. In this study, the combination effect of oncolytic HSV HF10 and Bevacizumab against experimental model of human breast carcinoma xenograft is evaluated.

**METHODS**

The VEGFA gene transcription and protein expression were measured in three human breast cancer cell lines (MCF-7, T47D and MDA-MB-231) by RT-PCR and ELISA. The MTT analysis was applied to evaluate the efficiency of the combination therapy in vitro. The effect on viral replication was assessed by PCR and plaque assay was performed for titering the virus under various doses of Bevacizumab. The advanced tumor model was formed by 12 female BALB/c nude mice which were implanted four pieces of 5 mm x 5mm x 5mm MDA-MB-231 tumor in the flank site. Meanwhile, the single tumor model included 28 female BALB/c nude mice which were implanted one piece of 5 mm x 5mm x 5mm MDA-MB-231 tumor in the right flank site. Bevacizumab group received 5μg/g Bevacizumab intraperitoneally twice a week for 2 weeks. The HF10 group of the single subcutaneous tumor model received single dose of $10^6$ pfu virus intratumorally on Day 1 and the HF10 group of the advanced subcutaneous tumor model received two doses of $10^6$ pfu virus per tumor intratumorally on Day 1 and Day 14. The combination group of the single subcutaneous tumor model received single dose of $10^6$ pfu virus intratumorally on Day 1 and 5μg/g Bevacizumab intraperitoneally twice a week for 2 weeks. The combination group of the advanced subcutaneous tumor model received two doses of $10^6$ pfu virus per tumor intratumorally on Day 1 and Day 14 and 5μg/g Bevacizumab intraperitoneally twice a week for 2 weeks. The tumor diameter and body weight were measured twice a week. On Day 3 and Day 36, the tumors which received treatment were collected and

observed respectively. Histopathological parameters were HIF1α, VEGFA, CD31 driven microvascular density, Caspase 3 and HSV-1 antigen.

**RESULTS**

Among the three candidate cell lines, MDA-MB-231 has the highest level of VEGFA expression, while T47D has the lowest level (Fig 1). The cytotoxic effect of HF10 was time- and dose- dependent (Fig 2a and 2d). Bevacizumab alone had no cytotoxic effect in vitro (Fig 2b and 2e) and the combination therapy had no additive effect in vitro (Fig 2c and 2f). MDA-MB-231 cell line grew faster (Fig 2g) and demonstrated more sensitivity (Fig 2a and 2d) than T47D cell line in vitro. Bevacizumab had no in vitro effect on viral replication on both MDA-MB-231 and T47D cell lines ($P>0.05$, Fig 3a, 3b, S-1a). In the in vivo study, the combination group has the smallest tumor volume comparing with other groups in both animal models ($P<0.05$, Fig 4, S-2, S-3). The combination therapy induces synergistic effect in vivo in both animal models (Table 1). Immunohistochemical staining for HSV-1 demonstrated diffuse virus distribution within the tumor in the HF10 group and the combination therapy group on day 3. The virus in the combination group replicated more efficiently than in the HF10 group, and a syncytial cytopathic effect (arrows) was clearly observed in the combination group (Fig. 5b). On day 36, high virus persistence within the tumor in the combination group was observed, while virus within the tumor in the HF10 group had been largely cleared (Fig. 5c). Hypoxia-inducible factor 1α (HIF-1α) was a transcriptional factor that regulated genes involved in response to hypoxia. HIF1α and VEGFA staining showed that the HIF1α and VEGFA gene were up-regulated after the treatment of Bevacizumab and HF10 and further up-regulated under the situation of combination therapy on day 3 (Fig. 5d and 5e). Neovascularization (CD31, arrows) was increased after the treatment of HF10 and was inhibited by the treatment of Bevacizumab and the combination on day 36 (Fig. 5f, 5g). We could hardly detect Caspase 3 staining in the control group, the Bevacizumab group and the HF10 group on day 3, however, a strong Caspase 3 staining was observed in the combination group probably due to the VEGF-mediated induction of the viral replication in endothelial cells and enhanced tumor hypoxia. (Fig. 5h).

**CONCLUSIONS**

Increased angiogenesis effect and limited viral distribution remain obstacles of oncolytic viral therapy. Anti-angiogenesis reagent such as Bevacizumab is considered to be effective to achieve better antitumor effect of oncolytic virus. Our results show that in the experiment models of human breast carcinoma xenograft, the combination of oncolytic virus HF10 and novel anti-VEGFA monoclonal antibody Bevacizumab induced a synergistic antitumor effect and therefore was superior to either therapy alone. The mechanism of the synergistic effect between Bevacizumab and HF10 was related to the increased vascular permeability to circulating virus, VEGF-mediated induction of viral replication in endothelial cells, innate immune-mediated

attack on virally infected vasculature, enhanced tumor hypoxia which stimulated the synthesis of virus beneficial proteins as well as expanded population of apoptosis. Our results suggested that in the treatment of human breast carcinoma, a combination therapy employing HF10 and Bevacizumab is warranted for further investigation. HF10 can be a promising agent in combination with Bevacizumab against cancer in the future.