LETTER TO THE EDITOR

Therapeutic Effects of LS-13 on Glioma *In Vivo* and *In Vitro*

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Glioma is the most common primary brain tumor, accounting for 40–50% of all malignant brain tumors [1]. In most cases, glioma is locally invasive. The resulting lack of boundary with surrounding healthy tissues is a major barrier for surgical resection. Treatment options include surgical resection followed by chemotherapy (e.g., with temozolomide) and irradiation. These treatments could prolong patient survival, but only to a limited extent, and at the cost of reduced life quality [2].

Angiogenesis is an important pathological progress in the growth, development, and invasion of solid tumors [3]. For glioma, new blood vessels must be formed to allow further growth when the tumor is 1–2 mm in diameter. As a result, interventions that inhibit angiogenesis could inhibit glioma growth [4]. LS-13 (molecular formula shown in Figure 1, from Tongli Pharmaceutical Technology Co., Ltd., Suzhou, China) is a novel small-molecule compound capable of inhibiting vascular endothelial growth factor receptor (VEGFR)-2 and VEGFR-3 kinases (IC_{50} at 3.1 and 3.4 nM, respectively; unpublished observation). We hypothesized that the inhibitory action of LS-13 on glioma is mediated by its effects on angiogenesis.

In the first step of the present study, we examined the potential effects of LS-13 on glioma, on the proliferation of

human umbilical vein endothelial cells (HUVEC). The results revealed concentration-dependent inhibition of LS-13 on HUVEC proliferation. In a scratch assays using cultured human U251 glioma cells, LS-13 significantly inhibited cell migration at a concentration of 10 μ M. In the next step, we conducted a transwell migration assay using HUVEC and U251 cells in logarithmic growth phase. Cells were starved overnight prior to the incubation with LS-13 in the upper chamber. The results showed that LS-13 incubation of 12 h inhibited the migration of HUVEC and U251 cells in a concentration-dependent manner (Figure 2).

In the next set of experiments, U251 cells were transplanted subcutaneously to 4-to 6-week-old nude mice before treatment with LS-13, when tumor size get to 100 mm³, then daily oral gavage at doses of 12.5, 25, 50, and 100 mg/kg/day over a 14-day period. The body weight and signs of stress were monitored daily. Tumor size was measured at different time points and estimated using an electronic caliper with the following equation: tumor size (cm³) = 1/2(length [cm]) $\times$ (width [cm])². The relative tumor volume (RTV) and relative tumor proliferation rate (T/C [%]) were then calculated. At doses of 50 and 100 mg/kg, LS-13 decreased RTV significantly ($P < 0.05$ vs. negative control group; Figure 3). A dose of 12.5 mg/kg, LS-13 decreased RTV significantly after Day 10 ($P < 0.05$), but at a higher dose of 25 mg/kg, LS-13 started to decreased RTV since Day 3 ($P < 0.05$; Figure 3). At a dose of 12.5 mg/kg, T/C was $>40\%$. At a higher dose of 25, 50, and 100 mg/kg, T/C was $<40\%$, suggesting that the lowest dose needed to inhibit glioma is 25 mg/kg. The body weight was not significantly reduced by LS-13 at any doses ($P > 0.05$).

In the last step of our experiments, we examined the effects of LS-13 (50 mg/kg/day via oral garage) in combination with doxorubicin (2 mg/kg twice every week, intravenously) over a 14-day period in nude mice bearing U251 glioma xenograft. RTV was significantly decreased by LS-13 treatment alone, doxorubicin alone,

Figure 1 Molecular formula of LS-13 with molecular weight of 473.50 Da.

Figure 2 The effect of LS-13 in different concentrations on the migration capacity of HUVEC and U251 cells through transwell test. (Transwell Lower Chamber cells).

Figure 3 Effects of LS-13 on tumor volume in nude mice bearing human glioma subcutaneously. (A: model group; B: the groups in dose of 12.5 mg/kg; C: the groups in dose of 25 mg/kg; D: the groups in dose of 50 mg/kg; and E: the groups in dose of 12.5 mg/kg and the groups in dose of 100 mg/kg).

as well as the combined treatments ($P < 0.05$ vs. negative control group). RTV in the combined treatment group was significantly lower than in the mice receiving LS-13 alone ($P < 0.05$), but not in the mice receiving doxorubicin alone ($P < 0.05$). T/C was 27.31% for LS-13 alone, 14.49% for doxorubicin alone, and 10%

for combined treatments. The body weight was not affected significantly by any of the treatment ($P > 0.05$).

Angiogenesis is a complex process that involves VEGF and its receptor VEGFR. Bevacizumab is an angiogenesis inhibitor and specifically VEGF-A inhibitor [5]. It has been approved by the US Food and Drug Administration (FDA) for recurrent glioblastoma. Bevacizumab does not prolong patient survival, but could improve quality of life considerably [6,7].

The VEGFR family includes VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 has high affinity for VEGF and strong kinase activity. It is primarily expressed in vascular endothelial cells and critically implicated in endothelial cell proliferation vascular permeability, neo-angiogenesis [8]. VEGFR-3 is mainly expressed in lymphatic endothelia, as well as on the surfaces of tumor vascular endothelial cells. VEGFR-3 is associated with the sprouting of blood vessels and the formation of new branches [9]. Simultaneous blockade of VEGFR-2 and VEGFR-3 is more effective in inhibiting tumors than VEGFR-2 blockade alone [8,9]. LS-13 inhibits the kinase activity of both VEGFR-2 and VEGFR-3. The results of this study support this mode of action.

Drug resistance is a formidable obstacle to tumor targeted therapy [10]. Drugs that target multiple molecular target and drug combinations are one strategy to minimize such a problem. In this regard, LS-13 has clear advantage: It targets both VEGFR2 and VEGFR3. We advocate further research of LS-13 as an addition to bevacizumab, the only approved targeted drug for glioma.

Conflict of Interest

The authors declare no conflict of interest.

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