Targeting Glioblastoma with an Anticancer MicroRNA

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ABSTRACT SUMMARY

Polyglycerol-Amine (PG-NH2) is a watersoluble polyglycerol-based hyperbranched polymer that accumulates in the tumor environment due to the enhanced permeability and retention (EPR) effect. PG-NH2 was recently developed by us and it showed promising results as an ideal delivery vehicle for antitumor biological agents.

We studied the expression targets and functional effects of several anticancer microRNAs (miRNAs) in human glioblastoma. Transfection of the miRNAs using our novel nanocarrier down-regulated their validated targets in several human glioblastoma cell lines. Transient transfection of the anticancer miRNA-PG-NH2 polyplex into glioblastoma cell lines strongly inhibited proliferation, cell cycle progression, and cell migration and tumor growth in vivo in a mouse model of human glioblastoma.

Together, these findings show that our novel nanocarrier is able to deliver anticancer miRNAs to glioblastoma cells and to suppress brain tumor growth by downregulating their validated targets.

INTRODUCTION

Glioblastoma (GBM) and other malignant gliomas are aggressive primary neoplasms of the brain that exhibit notable refractivity to standard treatments. MicroRNAs (miRNAs) are non-coding RNA molecules which act as post-transcriptional regulators of specific messenger RNA transcripts (mRNAs). miRNAs play major roles in normal developmental processes, and their deregulation significantly contributes to various aspects of carcinogenesis.

Small interfering RNAs (siRNAs) and microRNAs (miRNAs) on their own are not taken-up by most mammalian cells in a way that preserves their activity. In order to circumvent these limitations, we developed a cationic carrier system, which can strongly improve its stability, intracellular trafficking and silencing efficacy.

EXPERIMENTAL METHODS

PG-NH2 ability to neutralize siRNA/ miRNA negative charge was evaluated by an electrophoresis mobility shift assay and zeta potential measurement. The nanometric size of the carrier was measured with a Nanosight and visualized by atomic force microscopy (AFM). The cellular uptake of the siRNA-PG-NH2 polyplex was evaluated by confocal microscopy.

Human glioblastoma U87 cells and murine breast adenocarcinoma DA3 cells, labeled with luciferase, were injected subcutaneously into SCID mice and BALB/C mice respectively. We injected mice with luciferase siRNA or non-targeting-siRNA-PG-NH2 polyplex, first intratumorally and then systemically, and followed luciferase activity by non-invasive intravital bioluminescence imaging.

Cells were then challenged with our anticancer miRNA complexed with PG-NH2 at serial concentrations, and 72 hours later, proliferation was monitored by coulter counter. In addition, our miRNA overexpression and downregulation of its target genes was assessed by qPCR. Anticancer miRNA was also evaluated in a human glioblastoma mouse model. SCID mice bearing U87-mCherry glioblastoma tumors were treated with our anticancer miRNA-PG-NH2 polyplex or negative control miRNA-PG-NH2. Tumor volume progression was followed by caliper measurement and non-invasive intravital fluorescence imaging (Maestro™ CRI) up to the volume of 1200 mm3.

RESULTS AND DISCUSSION

We have recently developed polyglycerol-amine (PG-NH2), a water-soluble polyglycerol-based hyperbranched polymer with a range of mean hydrodynamic diameters of 10 to 40 nm. PG-NH2 accumulates in the tumor environment due to the enhanced permeability and retention (EPR) effect, and therefore, represents an ideal delivery vehicle for antitumor biological agents. PG-NH2 entrapment of siRNA neutralized its negative charge in a dose-dependent manner and significantly improved its cellular uptake (Figure 1).

Following biocompatibility and silencing efficacy studies in vitro the PG-NH2 delivery system was evaluated in vivo. In order to examine the potential feasibility of our novel siRNA/ miRNA-PG-NH2 based therapy, luciferase or non-targeting siRNA (NT-siRNA) complexed with PG-NH2 was injected first intratumorally (Fig. 2A) and then
systemically (Fig. 2B) to mice bearing luciferase-expressing tumors.

**Figure 1.** (A) Chemical structure of PG-NH$_2$ (Mw 10,000 g/mol). (B) Intracellular uptake of siRNA complexed with PG-NH$_2$. U87-Luc cells were incubated with Cy3-labeled siRNA (red), either naked or complexed with PG-NH$_2$ for 24 h. Actin filaments were stained with FITC-labeled phalloidin (green).

24 h after treatment, the luciferase activity was significantly reduced in the luciferase siRNA-injected group, as opposed to the NT siRNA-treated mice. We further continued with a novel anticancer miRNA. Transient transfection of our anticancer miRNA into glioblastoma cell lines strongly inhibited their proliferation, cell cycle progression, and migration. Next, we performed *in vivo* experiments and achieved a significant tumor growth inhibition effect in a human glioblastoma mouse model. While saline-treated mice lived for 30 days and mice treated with negative control miRNA (NC-miR) survived for 40 days, mice that were administered with our anticancer miRNA survived for 64 days.

We further characterized the synergistic effect of combining the miRNA polyplex with chemotherapy and achieved promising results on the proliferation and migration of the cells. We are currently working on a second generation of PG-NH$_2$ which combines the high efficacy of miRNA entrapment and delivery with a covalently-bound chemotherapy to be co-delivered to the tumor site. Together, these findings show that our miRNA-PG-NH$_2$ polyplex suppresses brain tumor growth by targeting its downstream effectors and inhibiting cell proliferation and migration, suggesting a key role of our anticancer miRNA in gliomas. The results also suggest that our anticancer polyplex could serve as a potential therapeutic agent for brain tumors.

**Figure 2.** (A) SCID mice bearing subcutaneous U87-Luc tumors were treated intratumorally with 10 mg/kg PG-NH$_2$ complexed with 2.5 mg/kg luciferase siRNA or NT-siRNA as control (n=3). (B) Balb/C mice bearing subcutaneous DA3-Luc tumors were treated systemically with 4 mg/kg PG-NH$_2$ complexed with 2.5 mg/kg luciferase siRNA or NT siRNA as control (n=3). Tumor volume was followed by intravital non-invasive bioluminescence imaging (Biospace).

**REFERENCES**


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