Reciprocal dormancy-promoting nanomedicine altering EGFR and TSP-1 for the management of glioblastoma

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ABSTRACT SUMMARY
A novel therapeutic approach for glioblastoma multiforme is introduced by a nanomedicine targeting multiple molecular targets based on the molecular fingerprint of tumor dormancy. We designed and synthesized a Thrombospondin-1 peptidomimetic (TSP-1 PM) and EGFR siRNA entrapped in polyglycerol-amine dendrimer. This combination therapy crosses the blood-brain barrier (BBB), accumulates at the intracranial tumor and reverts the angiogenic switch into a dormant-like state.

INTRODUCTION
Glioblastoma multiforme (GBM) is the most common form of primary brain tumors. It is one of the most aggressive and angiogenic forms of human cancers (1). With treatment (surgery, radiation and chemotherapy), the median survival is 12-14 months. For nearly all affected, the treatment remains palliative. Due to GBM’s infiltrating nature to the surrounding normal brain, complete removal of the tumor is impossible and hence results in high recurrence rate from residual tumor volume (2).

Tumor progression is dependent on a number of sequential steps, including initial tumor-vascular interactions and recruitment of blood vessels, known as the angiogenic switch, and an established interaction of tumor cells with their surrounding microenvironment. Failure of a microscopic tumor to complete one or more of these early stages, may lead to delayed clinical manifestation of the cancer and a state of stable non-progressing disease, named tumor dormancy. A dormant phase during tumor progression is highly prevalent, yet it is one of the most neglected areas in cancer research and the associated biological mechanisms are still mostly unknown (3). Dormant tumors are usually only a few millimeters in diameters and are, therefore, undetectable by most imaging modalities (4, 5). They can, however, switch to become fast-growing, clinically-apparent, and potentially lethal. Minimal residual disease contributes to tumor relapse, and constitutes fundamental clinical manifestations of tumor dormancy that together are responsible for the vast majority of cancer deaths.

RESULTS AND DISCUSSION
Analysis of molecular differences between fast-growing and dormant tumors revealed major dissimilarity in expression levels of TSP-1 and EGFR (Figure 1A). EGFR is overexpressed in fast-growing tumor cells (U-87-F and T98G-F) in gene and protein levels, and in tumor sections. In contrast, elevated levels of TSP-1 gene and secreted protein are found in dormant tumor cells (U-87-D and T98G-D). Therefore, we hypothesized that upregulation of TSP-1 signaling in parallel to downregulation of EGFR signaling, will induce a dormant-like state on GBM. We induced upregulation of TSP-1, using a TSP-1-peptidomimetic (TSP-1-PM), and downregulation of EGFR, using a dendritic nanocarrier entrapping EGFR siRNA. The ability of this combination therapy to reverse a fast-growing angiogenic phenotype of a tumor to a dormant avascular phenotype was evaluated.

EXPERIMENTAL METHODS
We have previously generated two GBM dormancy models in mice (6, 7). While the dormant avascular and fast-growing angiogenic tumor-forming cells share a similar growth rate in-vitro, we found profound differences in tumor growth patterns in-vivo. Furthermore, the cells differ in their angiogenic potential and in gene expression involved in angiogenesis regulation. Two of the major dissimilarities were thrombospondin-1 (TSP-1) and epidermal growth factor receptor (EGFR). The dormant tumor-generating cells express higher levels of TSP-1 and lower levels of EGFR compared to the fast-growing tumor-generating cells. TSP-1 is a key angiogenesis inhibitor, whose expression is lost during malignant transformation. EGFR is a modulator of tumorigenicity, thus considered an attractive potential target for therapy.

Figure 1. (A) Illustration of treatment rational design. (B) Polyglycerol-amine dendrimer entrapping EGFR siRNA. (C) Structure of TSP-1 PM.
Our novel dormancy-promoting nanomedicine penetrates via the BBB and accumulates in intracranial tumors following systemic administration (Figure 2A). Thirty percent co-localization was observed between Cy5-labeled dendrimer and mCherry-labeled tumor cells 10 min following i.v. injection. After accumulation at the tumor site, it internalized into GBM cells within 4 h (Figure 2B). 8 h following treatment, the nanomedicine escapes from the early endosome, as seen by decreased co-localization of siRNA with the dendrimer and the endosome. Only a small amount reaches the lysosome. 

Once escaped from the endosome and accumulates in the cytoplasm, the siRNA efficiently silences EGFR in GBM cells (Figure 3A). Decreased EGFR activity was also observed following treatment in a binding assay of fluorescently-labeled EGF which results in decreased cell viability (Figure 3B-C).

Finally, we tested the ability of our dormancy-promoting nanomedicine to revert the fast-growing angiogenic phenotype of GBM. Mice bearing established U-87-F tumors received TSP-1 PM (50 mg/kg daily) and PG-NH$_2$-siEGFR (2 mg/kg bi-weekly). The treatment exhibited anti-angiogenic and anti-tumorigenic activity. It remarkably decreased tumor volume by 99% compared with the control 25 days post treatment initiation, to a volume of 1 mm$^3$ (Figure 4A-B). The combination therapy also prolonged overall survival of mice by almost 50 days (Figure 4C-D). Immunohistochemistry analysis revealed reduced vasculature, increased aSMA and decreased VEGF expression in treated tumors. We concluded that TSP-1 PM in combination with EGFR-siRNA represents a promising treatment for advanced GBM promoting a dormant phenotype.

CONCLUSIONS

This work describes a novel approach for the treatment of GBM based on the promotion of a dormant phenotype using nanomedicine. In the model presented here, tumor dormancy is associated with impaired angiogenic potential, in particular high TSP-1 and low EGFR expression levels, and abnormal tumor vasculature (1-2). A nanomedicine inhibiting EGFR signaling via silencing with siRNA, simultaneously to induction of TSP-1 signaling, using peptidomimetic, reduced U-87-F angiogenic potential. Treated tumors reverted into a microscopic, dormant and avascular tumors. IHC revealed abundant VEGF levels and normalized tumor vasculature.

Our novel dormancy-promoting nanomedicine, targeting multiple molecular pathways, based on the molecular fingerprint of tumor dormancy, provides a signature treatment towards regression of aggressive glioblastoma into a dormant asymptomatic disease.

REFERENCES

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