Sustained Released Anti-angiogenic Thrombospondin Mimetic Peptide Induces an Angiogenesis-Dependent Dormant-Like Phenotype of Glioblastomas

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ABSTRACT SUMMARY
A novel therapeutic approach for glioblastoma multiforme is introduced by inducing a dormant-like phenotype on cancer cells and depriving them from angiogenesis. We evaluated the ability of Thrombospondin-1 (TSP-1)-peptidomimetic to regress the fast-growing angiogenic phenotype of U-87 MG to a dormant avascular phenotype. TSP-1-peptidomimetic attenuated tumor progression, reduced VEGF-induced hyperpermeability and normalized tumor vasculature.

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 cancer (1). With treatment (surgical resection, 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 very 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 (i.e., the angiogenic switch), and an established interaction of tumor cells with their surrounding microenvironment and its components. 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 (i.e., 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). It is present as one of the earliest stages in tumor development, as micro-metastases in distant organs, and as minimal residual disease left after surgical removal or treatment of primary tumors (referred to as minimal residual disease). Dormant tumors are usually only a few millimeters diameter in size and are, therefore, undetectable by most imaging modalities currently in use (4, 5). They can, however, switch to become fast-growing, clinically-apparent, and potentially lethal. Minimal residual disease contributes to the occurrence of relapse, and constitutes fundamental clinical manifestations of tumor dormancy that together are responsible for the vast majority of cancer deaths.

EXPERIMENTAL METHODS
We have identified and isolated a dormant tumor-generating clone, derived from the aggressive tumor-forming U-87 MG human glioblastoma cell line (6), using gene expression signature of dormant tumors (7). The two cell lines exhibit profound differences in their angiogenic potential and gene expression involved in angiogenesis regulation. One of the major dissimilarities was found in thrombospondin-1 (TSP-1) expression levels. The dormant avascular tumor-generating cell line (U-87-D) express significantly higher levels of TSP-1 compared to the fast-growing angiogenic tumor-generating parental cell line (U-87-F). It has been previously demonstrated that TSP-1 is a key endogenous angiogenesis inhibitor. Therefore, it has been established as an attractive potential therapy for angiogenesis-dependent diseases.

In this study, we evaluated the ability of TSP-1-peptidomimetic to regress the fast-growing angiogenic phenotype of U-87-F to the dormant avascular phenotype of U-87-D. Mice bearing established U-87-F tumors received daily treatment of TSP-1-peptidomimetic (50 mg/kg/day) by slow-release ALZET® osmotic pump (0.5 μl/h).

RESULTS AND DISCUSSION
Identification of dormant tumor-generating clone was based on gene expression analysis of single cell-derived clones from U-87 MG cell line. TSP-1 expression level was chosen as a primary indicator for slow tumor growth. Three clones were selected for further investigation for expression of genes previously shown to be upregulated in
dormant tumors and upregulated in fast-growing tumors (Figure 1).

Tumor growth kinetics of U-87 MG-derived clones were evaluated in SCID mice and a correlation with TSP-1 expression was observed. While the parental cell line (U-87-F) and the dormant clone (U-87-D) share similar growth rate in vitro, we found profound differences in tumors' growth pattern when injected into mice (Figure 2). U-87-F established palpable and vascularized tumors only few days following inoculation, whereas U-87-D-generated tumors remained at a small size for more than 100 days.

Since TSP-1 is a key angiogenesis inhibitor and the two cell lines exhibit profound differences in gene expression involved in angiogenesis regulation (6), TSP-1-peptidomimetic is a good candidate for therapy. In order to best imitate the clinical pharmacokinetic setting, TSP-1-peptidomimetic was administered (50 mg/kg/day) by systemic subcutaneous slow-release ALZET® osmotic pump.

TSP-1-peptidomimetic attenuated tumor progression in treated mice compared with control mice (Figure 3). Immunohistochemistry analysis of treated tumors revealed reduced abnormal vasculature, increased αSMA expression and decreased VEGF expression. Therefore, we concluded that slow-released TSP-1-peptidomimetic in combination with targeted chemotherapy may present a promising treatment for progressive GBM.

**CONCLUSIONS**

This work describes a novel approach for the treatment of glioblastoma multiforme based on the promotion of a dormancy phenotype. In the model presented here, tumor dormancy is associated with impaired angiogenic potential, in particular high TSP-1 expression levels, and abnormal tumor vasculature (1-2). Induction of TSP-1 signal transduction using peptidomimetic reduced U-87 MG angiogenic potential and VEGF levels, normalized tumor vasculature and finally inhibited tumor growth.

Controlled slow-released TSP-1 peptidomimetic in combination with other signature dormancy-inducing factors and chemotherapy could provide an improved treatment towards regression of aggressive glioblastoma into a dormant-like state.

**REFERENCES**