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14. ABSTRACT

Brain metastasis occurs in ~30% of metastatic breast cancer patients. The prognosis is extremely poor, with a median survival of 4-6 months even with aggressive treatment. Thus, there is an urgent need to develop new treatments that target brain metastases. Radioimmunotherapy (RIT) is a targeted therapy that uses radiolabeled antibodies against tumor-specific antigens to treat lymphoma patients. However, success of RIT in the therapy of solid tumors has generally been limited due to heterogeneous tumor expression of the target antigens and cross-reactivity with normal cells. In preliminary studies, we have demonstrated that phosphatidylserine (PS) is exposed exclusively on tumor vascular endothelium of brain metastases in mouse models. A novel PS-targeting antibody, PGN635, a fully human monoclonal antibody, was used to target exposed PS in the brain metastases. Our data show that PGN635 binds specifically to tumor vascular endothelial cells in multi-focal brain metastases throughout the whole mouse brain. Vascular endothelium in normal brain tissues is negative. Furthermore, pretreatment with 10Gy of whole brain radiation significantly increased PGN635 binding to tumor vascular endothelial cells and tumor cells by increasing their exposure of PS. Vasculature in irradiated normal brain remained negative for exposed PS.

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Introduction

Brain metastasis is the most common intracranial malignancy in adults. The prognosis is extremely poor, with a median survival of 4-6 months even with treatment. Due to the high incidence of multiple lesions and limited leakage of most chemotherapeutic agents through blood brain barrier (BBB), the standard care for these patients involves whole brain radiotherapy (WBRT). WBRT is often associated with neurological complications that preclude delivery of the sufficient dose to tumor lesions. Radioimmunotherapy (RIT) using radiolabeled monoclonal antibodies against tumor-specific antigens offers the opportunity to selectively irradiate tumor cells while sparing normal tissues. We have applied a phosphatidylserine (PS)-targeting antibody, PGN635, to study brain metastases in mouse models of breast cancer. PS is an integral membrane phospholipid that is maintained on the inner leaflet of the plasma membrane. It becomes externalized under stressful conditions or when cells undergo programmed cell death. PS exposure is a conserved immunosuppressive signal serves as an immune checkpoint to prevent the induction of autoimmunity to dying cells. PGN635 and other antibodies developed by our group that target PS inhibit this immune checkpoint and stimulate a productive immune response. The overall goal of this proposal is to test the hypothesis that PS-targeted radioimmunotherapy provides an effective and safe treatment for brain metastases.

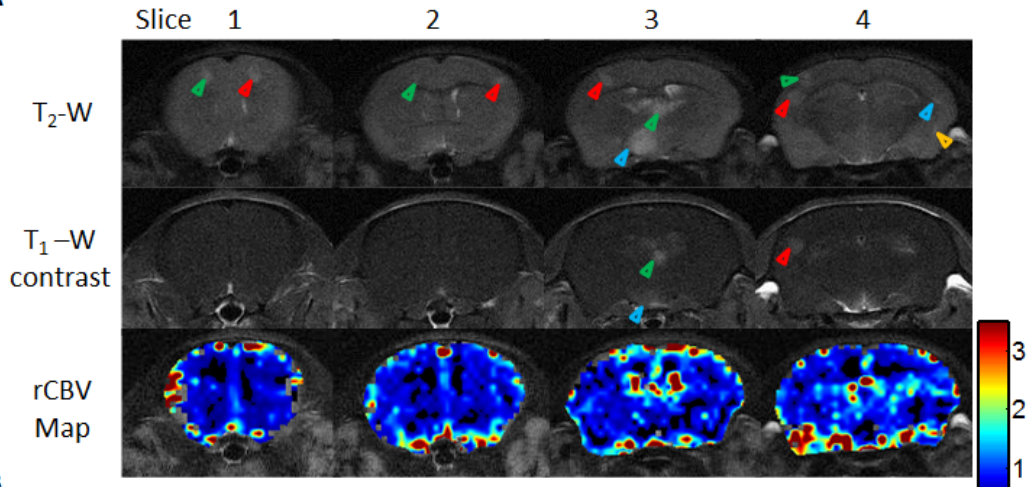
Prior work from the Thorpe laboratory has demonstrated that PS is externalized on vasculature in the tumor microenvironment (1). Importantly, therapy increases the exposure of PS on endothelial cells and tumor cells and the localization of anti-PS antibodies to the tumor microenvironment. The anti-PS antibodies (e.g., PGN635) once bound block PS induced immune suppression and drive activation of an innate immune response against the tumor. This response includes antibody dependent cellular cytotoxicity (ADCC) which results in targeting of PS positive cells for immune mediated destruction. We have shown recently in murine models of prostate cancer that antibody mediated blockade of PS results in striking changes in immune cell phenotype and potent anti-tumor efficacy (2).

Results

The current proposal has 3 tasks.
Task 1. To study PS exposure on tumor vasculature and tumor cells of breast cancer brain metastasis and determine if exposed PS is increased by radiation.

As part of the project, Dr. Zhao's laboratory established a model of brain metastasis where breast tumor cells (e.g., MDA-MB-231/BR cells) are seeded in the brain via intracardiac injection. His laboratory also demonstrated that brain metastases established in this manner can be visualized via MRI. (Task 1; Parts a & b) (see Figures 1 & 2)

A



B

	slice 1		slice 2		slice3			slice4				Mean
Mets	0.72	0.60	1.19	0.45	0.54	1.12	0.68	0.57	1.04	0.83	0.56	0.76±0.26
Contralateral	1.09	1.13	0.58	0.78	0.88	1.61	0.41	0.81	0.97	1.04	1.71	1.00±0.39

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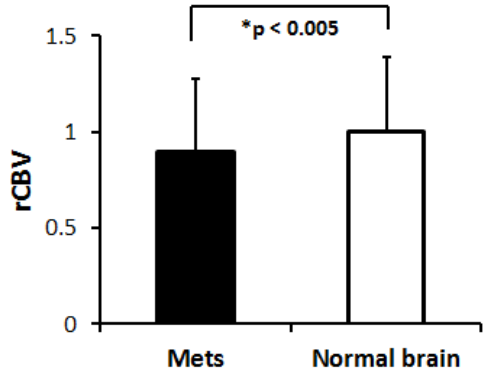

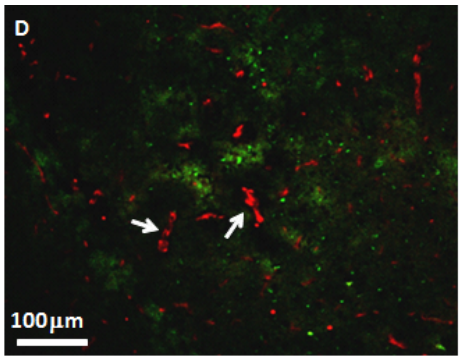

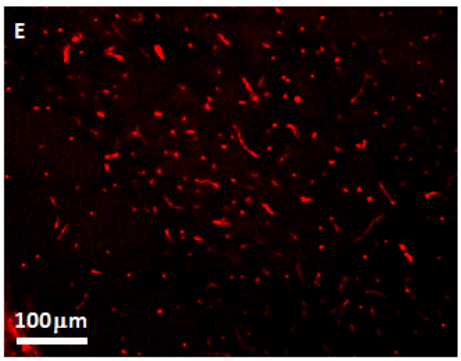

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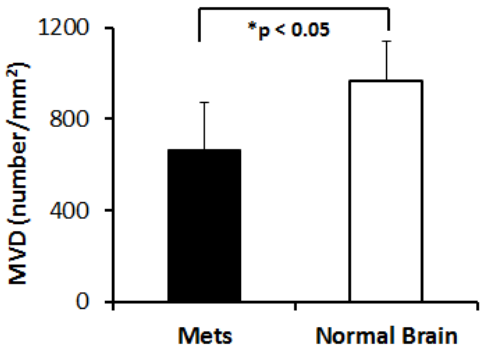

Fig. 1 MRI evaluation of tumor vascularity of brain metastases and correlating with histological studies. A. Four weeks after intracardiac injection of 231Br cells, T_2-weighted MRI revealed multiple high signal intensity lesions (arrowheads) on four consecutive coronal sections of a representative mouse brain. Only a few of the lesions (arrowheads) were enhanced on T_1-weighted post contrast images, one (blue arrowhead in the MRI section 3) of which showed partial enhancement, indicating intratumoral heterogeneity of BTB disruption. rCBV maps of the four sections were generated and overlaid on the T_2-weighted images. **B.** The rCBV values of the metastatic lesions and their contralateral normal brain were obtained and summarized in the table. Note the color presented in the table coincides with the color of arrowhead on each of the MR images. Most of metastatic lesions had lower rCBV values than their contralateral counterparts of normal brain. **C.** Statistical analysis of rCBV in a total of 212 lesions of 9 animals obtained from the last follow-up MRI showed significantly lower rCBV of the metastatic tumors with a mean value of 0.89 ± 0.03 (s.e.), compared to the contralateral normal brain (mean = 1.00 ± 0.03; $p < 0.005$). **D.** Anti-CD31 staining was performed on a brain section bearing metastases. A cortical lesion (~ 600 µm in diameter) was depicted with green fluorescence (GFP). Microvessels (red) within the lesion appeared less dense, as compared to abundant fine vessels in the contralateral normal brain tissues (**E**). Some of the tumor vessels were irregular in shape and larger in diameter (arrow). **F.** Quantitative data of MVD showed a significantly lower MVD in brain metastases versus contralateral normal brain (mean = 669 ± 201/mm^2 vs. 965 ± 177/mm^2; $p < 0.05$).

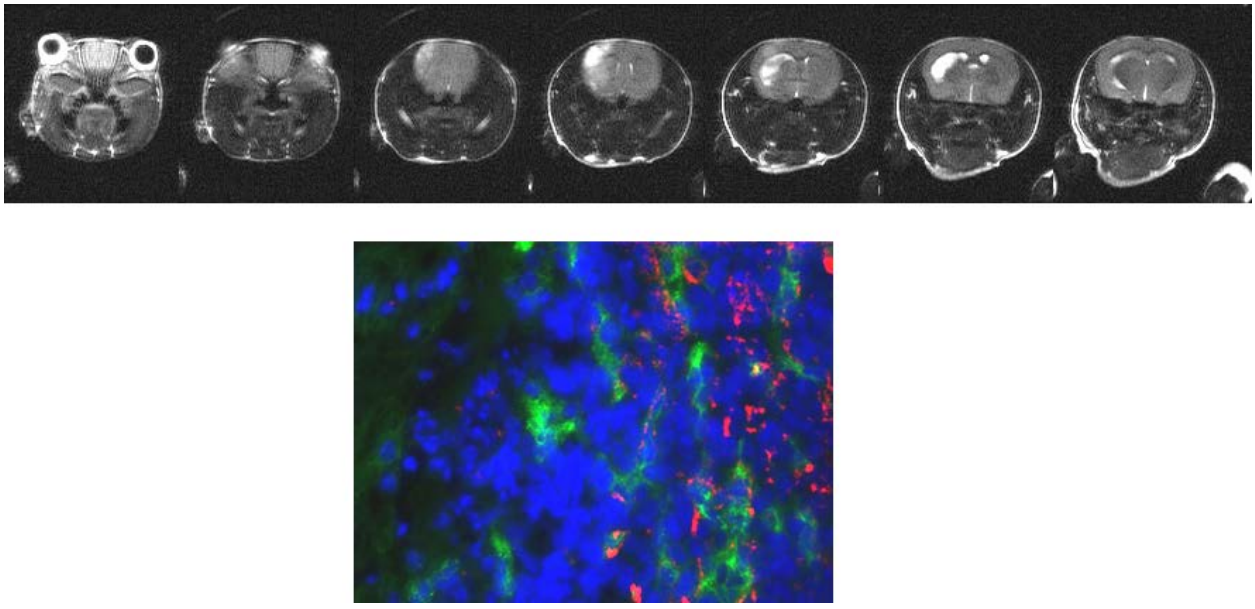

Fig. 2 Enhanced PS exposure after WBRT. Top. T2-weighted MRI clearly revealed a high-signal intensity tumor lesion. **Bottom.** After a single dose of 10 Gy WBRT, immunohistochemical staining was conducted on the tumor specimen with CD31 (green), PGN635 (red) and Dapi (blue). In addition to vascular exposed PS (orange), massive tumor cells were detected with PS exposure.

In collaboration with Dr. Zhao we demonstrated that PS is exposed on the vasculature of breast cancer brain metastases and have evaluated microvessel density (MVD) and hypoxia.

We obtained tissue from mice bearing brain metastases that were subjected to WBRT. These animals were injected iv with PGN635 4 hours prior to sacrifice. The tissue was processed for immunohistochemical evaluation of PS exposure, MVD, markers of hypoxia, and cell proliferation and apoptosis. (Task 1; part d).

Task 2. To radiolabel mch635 and evaluate its biodistribution and pharmacokinetics in breast cancer brain metastasis.

We produced and provided PGN635 F(ab')2 to Dr. Zhao who will provide an update on the imaging properties of the radiolabeled antibody fragment in his final progress report.

Task 3. To evaluate radioimmunotherapy of breast cancer brain metastases.

Key Research Accomplishments (consistent with Dr. Zhao's forthcoming report)

- Extensive imaging studies of the intracardiac model of breast cancer brain metastasis with various brain-tropic metastatic breast cancer cells including MDA-MB231Br-EGFR, MCF7Br-Her2 and syngeneic 4T1 cells.

- MRI was applied to evaluate vascular perfusion and BTB permeability of brain metastases. Our data showed significantly lower tumor perfusion in brain metastases as compared to the contralateral normal brain, and less than half of brain metastases containing disruptive blood-tumor-barrier (BTB), which correlated well with histological analyses.

- Immunohistochemical studies show PS exposure is specifically located on tumor vascular endothelial cells of brain metastases while the normal vessels surrounding the metastases lack of exposed PS, suggesting that PS can serve as a brain metastasis-specific biomarker.

- Whole brain radiation (WBRT) induced significantly more PS exposure on both tumor vascular endothelial cells and tumor cells of brain metastases.

- PS-targeting antibody, PGN635F(ab')2 has been successfully conjugated with radioisotope, enabling in vivo PET imaging for sensitive detection of brain metastases.

Challenges

Major challenge were faced during this project. The first significant challenge was the passing the sudden passing of Dr. Phil Thorpe in March of 2013. This caused a significant delay in progress on the project. A second significant challenge was the move of Dr. Dawen Zhao from UTSW to Wake Forest in late summer of 2015.

Reportable outcomes

Recent PS related Publications: There are 3 manuscript submitted relevant to PS targeting from our group.

Cheng* X, Li* L, Thorpe PE, Yopp AC, **Brekken RA** and Huang X. Antibody-mediated blockade of phosphatidylserine enhances the antitumor effect of sorafenib in hepatocellular carcinoma xenografts. Submitted. * equal contribution

Freimark B, Gong J, Ye D, Gray M, Nguyen, Yin S, Shuler-Hatch M, Hughes C, Schroit AJ, Hutchins JT, **Brekken RA** and Huang X. Antibody-mediated phosphatidylserine blockade enhances the anti-tumor response to CTLA-4 and PD-1 antibodies in melanoma. Submitted

Birge RB, Boeltz S, Kumar S, Carlson J, Wanderley J, Calianese D, Barcinski MA, **Brekken RA**, Huang X, Hutchins JT, Freimark B, Empig C, Mercer J, Schroit AJ, Schett F and Herrmann M. Phosphatidylserine (PS) is a global immunosuppressive signal in efferocytosis, infectious disease and cancer. submitted

Conclusion

During this project we have worked with Dr. Dawen Zhao to establish murine models of brain metastasis. Dr. Zhao has evaluated these models using MRI techniques that have enabled visualization of individual lesions in the brain. We have exploited the specificity of PGN635, a human anti-PS antibody to visualize externalized PS on the vasculature and tumor cells in brain lesions. PGN635 specifically localized to the vasculature in metastases and did not react with blood vessels in normal brain tissue. We conclude that PS, as expected is an excellent marker of tumor vasculature and anticipate that therapy studies combining radiation and anti-PS will provide potent therapeutic efficacy.

References

1. He J, Yin Y, Luster TA, Watkins L, Thorpe PE. Antiphosphatidylserine antibody combined with irradiation damages blood vessels and induces tumor immunity in a rat model of glioblastoma. Clin. Can. Res. 2009 15: 6871-6880. PMID: 19887482.
2. Yin Y, Huang X, Lynn KD, Thorpe PE. Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Can. Immunol. Res*. 2013. 1: 256-268.