Uncovering a Novel Role for HLA-G in Brain Metastases

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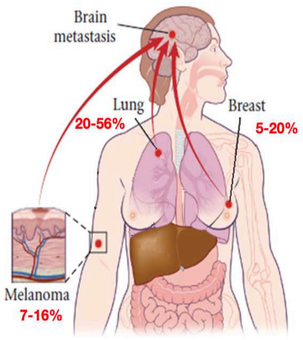
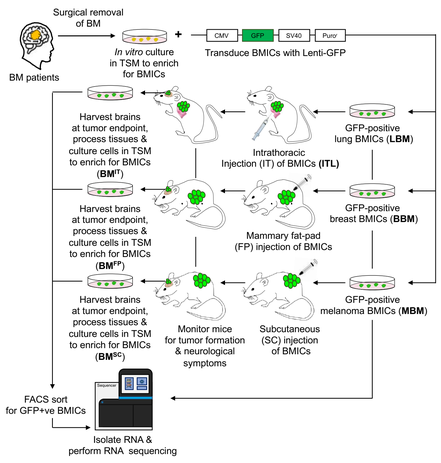
ABSTRACT



Brain metastases (BM) are the most common brain tumor in adults with the three major sources being primary lung, breast, and skin (melanoma) cancers. Current BM treatment regimens unfortunately do not effectively eradicate the disease resulting in reduced median survival times (~12 months) in treated patients. This indicate the need for more effective therapies against BM, which can be informed by a better understanding of the molecular biology of the disease especially at its early stage.

STUDY DESIGN / OBJECTIVES

Using our murine, orthotopic, patient-derived xenograft (PDX) models of lung-BM (LBM), breast-BM (BBM) and melanoma-BM (MBM), we captured brain metastatic initiating cells (BMICs) at the early phase of the BM cascade, which we termed "pre-metastatic". Transcriptomic analyses of these cells in comparison with their parental (macro-metastatic) BMICs identified several genes that are commonly upregulated only in pre-metastatic BMICs including HLA-G. ***Herein, we aimed to determine the functional role of HLA-G in BM***.



RESULTS

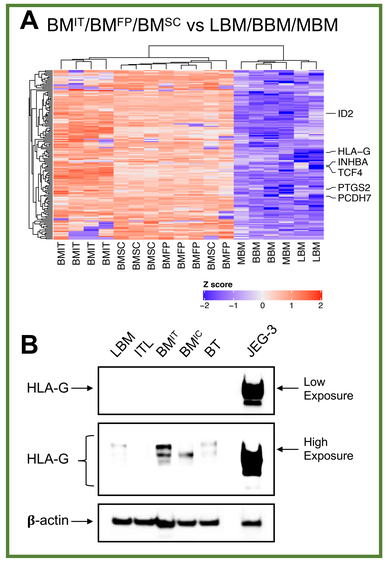


Figure 1. HLA-G is highly expressed in pre-metastatic BMICs at both the transcript and protein levels. A) Heatmap showing the top gene hits with a 3-fold or higher transcript expression and a false discovery rate (FDR) of <0.05 across all three pre-metastatic (BM^{IT}, BM^{FP} and BM^{SC}) subsets compared to their parental macro-metastatic counterparts (LBM, BBM and MBM respectively). **B)** Western blot analysis revealed a higher expression of HLA-G at the protein level in BMICs obtained during the early (pre-metastatic) phase of the lung-BM cascade compared to BMICs obtained from parental (LBM), orthotopic (ITL) or during the later (micro-/macro-metastatic) stages (BM^{IC} and BT) of the lung BM cascade. The choriocarcinoma cell line JEG-3 is used as a positive control for HLA-G expression. β-actin is used as a loading control. Experiments were performed independently at three times.

RESULTS

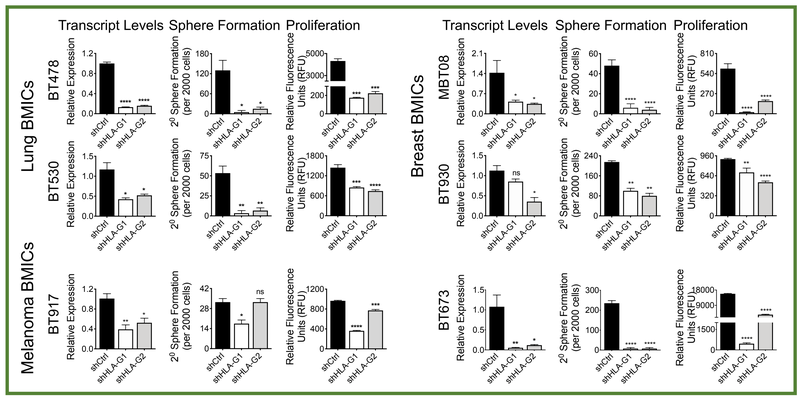


Figure 2. HLA-G loss attenuates the sphere formation and proliferation of lung, breast and melanoma BMICs. Patient-derived lung, breast and melanoma BMICs were transduced with control (shCtrl) or two HLA-G-specific short hairpins (shHLA-G1 and 2) and subjected to transcript (qRT-PCR) analysis, secondary sphere formation and proliferation assays to determine the effect of HLA-G depletion on the secondary sphere formation and proliferation of lung, breast and melanoma BMICs. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

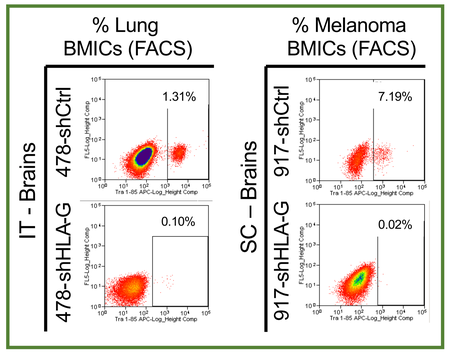


Figure 3. HLA-G loss impedes the accumulation of BMICs during the early (pre-metastatic) phase of the BM cascade. NOD SCID Gamma (NSG) mice (n = 6) were injected orthotopically (intrathoracically or subcutaneously) with either shCtrl or the most efficient shHLA-G lung or melanoma BMICs respectively. At endpoint, each mouse brain were harvested, minimally cultured and subjected to flow cytometry to capture pre-metastatic lung and melanoma BMICs.

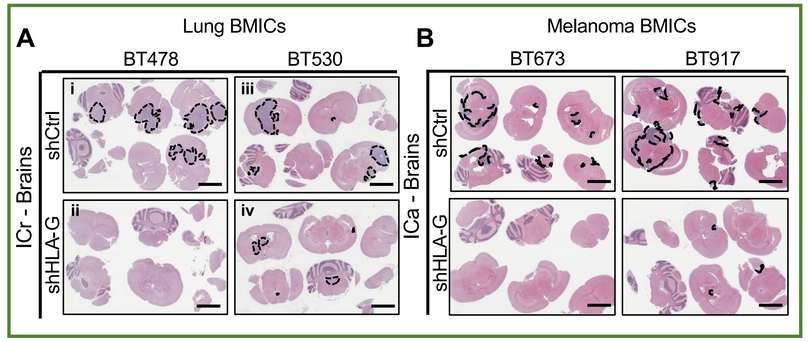


Figure 4. HLA-G loss reduces the ability of BMICs to form secondary brain tumors *in vivo*. shCtrl and shHLA-G lung **(A)** and melanoma **(B)** BMICs were injected directly into the brains of NSG mice and allowed to engraft and form tumors. At endpoint, the brains of each mice group were harvested, formalin fixed, paraffin embedded and subjected to hematoxylin & eosin (H& E) staining. All the shCtrl lung and melanoma BMICs formed large tumors in the brains of injected mice as seen from the emboldened broken enclosed lines. In contrast, shHLA-G lung and melanoma BMICs formed few to no tumors in the brains of injected mice (n = 5 for BT478 and BT673 or n = 3 for BT530 and BT917).

RESULTS

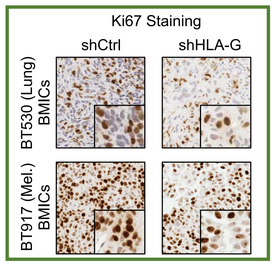


Figure 5. HLA-G depletion attenuates BM formation by reducing the proliferation of BMICs *in vivo*. BT530 (lung) and BT917 (melanoma) shCtrl and shHLA-G xenograft/brain tumor tissues were stained for the proliferation marker Ki67. Both BT530 and BT917 shHLA-G tumor cells displayed limited Ki67 staining and thus reduced proliferation when compared to the prominent Ki67 staining pattern seen in BT530 and BT917 shCtrl tumor cells.

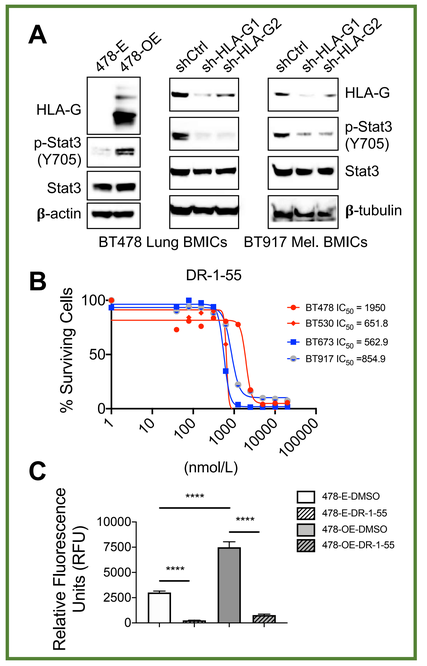


Figure 6. HLA-G promotes BMIC proliferation via the STAT3 signaling pathway. A) BT478 (lung) BMICs were transduced to overexpress (OE) HLA-G while both BT478 and BT917 (melanoma or mel.) BMICs were transduced to knockdown (shHLA-G1 and 2) HLA-G expression. Control (E and shCtrl) and either OE or shHLA-G1 and 2 lung and mel. BMICs were then subjected to western blot analysis of phosphor(p)-Stat3, which showed an increase or decrease in p-Stat3 levels upon HLA-G overexpression or knock-down respectively. **B)** Dose-response curve of lung (BT478 & BT530) and mel. (BT673 & BT917) BMICs treated with the STAT3 inhibitory drug – DR-1-55. **C)** Proliferation assays conducted on BT478 Ctrl (E) and OE BMICs show a decrease in the proliferation of lung BMICs upon STAT3 inhibition even in the presence of HLA-G overexpression. **** $p < 0.0001$.

CONCLUSIONS

Brain metastatic cells from various primary cancers including lung and breast cancers and melanomas display common genetic signatures at the early stage of the BM cascade.

One such gene – HLA-G is essential for BM establishment from primary lung and melanoma cancers, functioning via the STAT3 signaling pathway to promote BMIC proliferation *in vitro* and *in vivo*. HLA-G could be potentially targeted for BM treatment.

KEY REFERENCES

1. Achrol AS, et al. Brain metastases. *Nat Rev Dis Primers*. 2019 Jan 17;5(1):5.
2. Singh, M. et al. Therapeutic Targeting of the Premetastatic Stage in Human Lung-to-Brain Metastasis. Cancer Res 78, 5124-5134, doi:10.1158/0008-5472.CAN-18-1022 (2018).

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