An *in vivo* functional genomics screen to identify novel drivers of lung-to-brain metastasis

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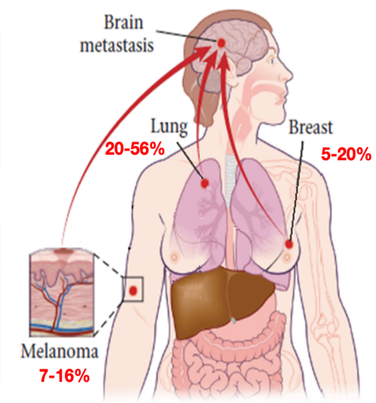
Abstract

Brain metastases (BM), the most common tumors of the central nervous system, occur in approximately 20% of primary adult cancers[1]. In particular, 40% of patients with non-small cell lung cancer (NSCLC) develop BM, with survival of only 4-11 weeks once diagnosed without treatment, and 16 months with treatment[2]. As systemic therapies for the treatment of NSCLC are becoming increasingly effective at controlling primary disease, patients are ironically succumbing to their BM. This highlights a large unmet need to develop novel targeted therapies for the treatment of lung-to-brain metastases (LBM). We hypothesize that an *in vivo* functional genomic screen will identify novel genes that drive LBM. To do this, we developed a murine, orthotopic, patient-derived xenograft (PDX) model of LBM using patient-derived NSCLC cell lines. This PDX model of LBM enables the use of fluorescent and bioluminescent *in vivo* tumor cell imaging to track primary tumor progression in the lung and the formation of both intra- and extra-cranial metastases. We propose to perform *in vivo* functional genomic screening to identify novel drivers of primary lung tumor formation as well as LBM. This platform will lead to potential therapeutic targets to prevent the formation of LBM and prolong the survival of patients with NSCLC.

Significance and Hypothesis



NSCLC most commonly metastasizes to the brain irrespective of patient sex and is the most common cause of BM in men. Most systemic therapies for the treatment of NSCLC have poor penetration into the brain and are thus largely ineffective at treating LBM. Furthermore, similar to primary brain tumors, the clonal heterogeneity of BM make the current standard of care therapies inadequate and unable to address issues of treatment resistance. In addition, the inherent signals that drive LBM are likely different from those that drive primary tumor formation, thus therapies targeted against a patient's primary tumor may not be effective at treating their BM. This is exemplified by reports that genetic profiling of patient lung adenocarcinoma tumors links the presence of driver mutations in *EGFR* and *ALK* gene rearrangement to increased formation of BM[3,4]. We have previously established various animal models of BM in our lab[5], and being equipped with the expertise in preclinical murine models, we hereby propose a robust and novel model that is suitable for large-scale genomics screening.

Hypothesis: We hypothesize that by harnessing the power of *in vivo* functional genomics screening in a relevant murine PDX model of LBM, we can gain insight into the mechanism(s) of LBM formation and identify druggable targets with translational potential to improve the survival of NSCLC patients.

Methods and Results

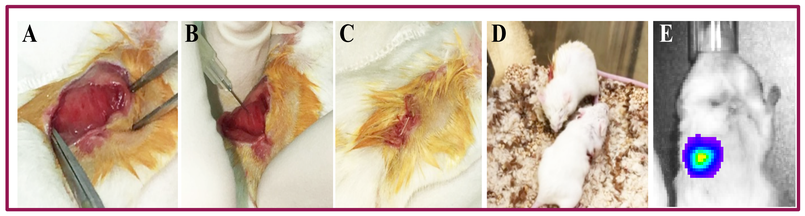


Figure 1. Schematic of our method for generating an orthotopic PDX model of NSCLC. A) Right-sided thoracotomy exposes the rib cage and underlying pleural cavity and lung. **B)** Injection of GFP-Luciferase-expressing (GFP-Luc) patient-derived NSCLC cells into the right middle lung lobe of a SCID mouse. **C)** Thoracotomy wound closure using sutures. **D)** Post-operative recovery without any morbidity and mortality. **E)** Bioluminescent intravital imaging (IVIS) three days post-tumor induction shows focal intrathoracic tumor signal.

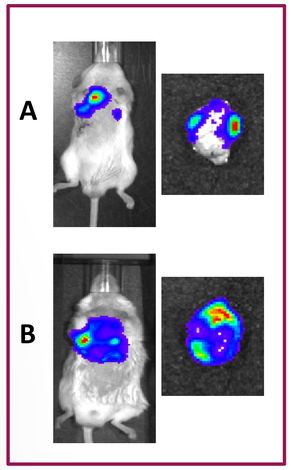
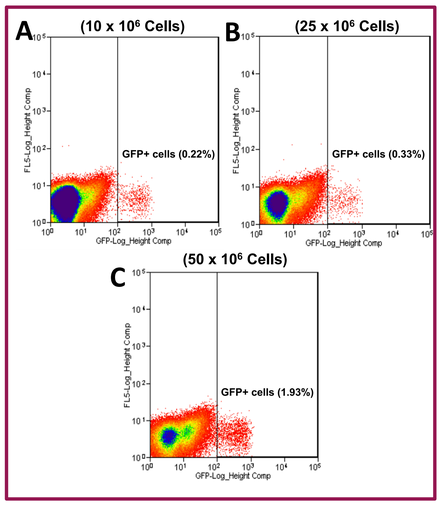


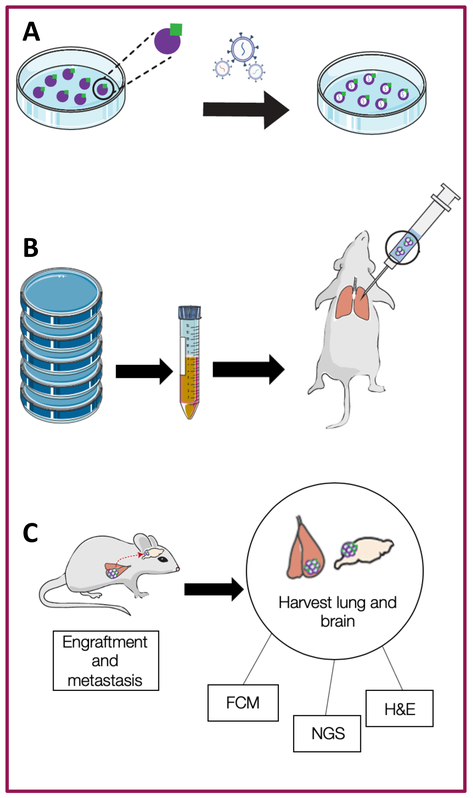
Figure 2. *In vivo* bioluminescence imaging of intrathoracically injected mice displays the successful modelling of LBM. A) A SCID mouse was injected with 10 x 10^6 GFP-Luc NSCLC tumor cells. Three days post-injection, a clear focal lung signal was visible (left panel). The brain was harvested after one month and imaged ex-vivo using IVIS to visualize BM formation. Note the presence of focal tumor signal in the hemispheres of the brain (right panel). **B)** A SCID mouse was injected with 50 x 10^6 GFP-Luc NSCLC tumor cells. Three days post-injection, the mouse exhibited higher primary tumor signal on IVIS than the mouse in A, with *ex vivo* imaging of the brain at one month post-injection showing well-defined bioluminescent tumor signal in the frontal cortex and well as in the cerebellum. These patterns of BM formation recapitulate those that are seen in human patients.

Figure 3. Flow cytometric analysis of mouse lungs harvested one week post-injection. In order to test the range of cell numbers able to be injected through our orthotopic mouse model and form a viable lung tumor, we tested tumor induction using different cell numbers. The following numbers of GFP-Luc NSCLC cells were injected into the lungs of SCID mice: **A)** 10 x 10^6, **B)** 25 x 10^6, and **C)** 50 x 10^6. Lung tissue and tumors were harvested after one week and cell counts were recorded ranging from 80 to 90 million cells per tumor. Cells were analyzed using flow cytometry for the GFP+ population and the final human cell population extrapolated based on the GFP+ percentage.



Methods and Results

Figure 4. Schematic of a potential *in vivo* functional genomic screen using patient-derived NSCLC cells in our PDX model of LBM. A) Lung tumor cells derived from fresh patient tumor tissue are processed into single cell suspension and infected with the GFP-Luciferase construct in order to enable *in vitro* and *in vivo* imaging. After the cells with the highest level of GFP expression are isolated and expanded, they are infected with any genomic perturbation library (shRNA, CRISPR, cDNA, etc.) *in vitro* to appropriate representation. **B)** Cells are then expanded *in vitro*, selected using the selectable marker over a period of time, and injected intrathoracically into the lungs of immunocompromised mice. **C)** Tumor growth and metastases are tracked using bioluminescence and organs harvested for downstream purposes to identify and other downstream applications.



Conclusion and Future Directions

By developing a patient-derived mouse model of NSCLC that can recapitulate patterns of BM as those seen in human patients, we can perform downstream *in vivo* functional genomics screening to identify novel drivers of LBM. We aim to interrogate the molecular and genetic underpinnings of the lung-to-brain metastatic cascade to inform the potential tailoring of treatments for NSCLC patients. This approach can theoretically be adopted for other PDX models of BM.

Key References

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TEAM KELSEY
BoxRun "Running for the Fight"
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SABCR