The SRG Rat, an immunodeficient model for orthotopic glioblastoma, diffuse intrinsic pontine glioma (DIPG) PDX, and intracranial metastatic breast cancer PDX tumors

#PB007

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Introduction

Immunodeficient rodent models are vital preclinical models, allowing xenografting of human cells and tissues for drug efficacy and tolerability testing in a human-like disease setting. Historically, immunodeficient mice have been the standard species for cancer xenografts. We created a Sprague Dawley Rag2 -/-, Il2rg -/- rat (SRG Rat®) that readily supports engraftment of a variety of human cells, tissues, and tumors. The SRG rat lacks B, T, and NK cells and is more immunodeficient than the Nude rat.

Here we demonstrate the utility of the SRG Rat in supporting intracranial growth of cell and patient derived human cancers that grow in the brain. We inoculated female SRG rats with human glioblastoma cell lines U87MG-luc and U118MG-luc to establish cell line derived xenograft (**CDX**) tumors in the striatum of the rat brain. Additionally, patient derived xenograft (**PDX**) models from glioblastoma and metastatic breast cancer tumors in the brain were isolated, briefly cultured, and inoculated into the striatum of male SRG rats. Finally, PDX models were established from diffuse intrinsic pontine glioma (DIPG) patients, a very rare but lethal childhood cancer. All tumor growth was monitored via in vivo imaging.

These data confirm that the SRG Rat is an excellent host for studying many different types of orthotopic human brain cancer. Due to its larger size, ability to support a higher tumor burden when compared to mouse models, and permissiveness to human cancer cells, these data demonstrate that the SRG Rat has a high utility for studies requiring intracranial orthotopic tumor implantation. As the most immunodeficient rat commercially available, the SRG Rat supports pre-clinical testing of brain cancer therapeutics in a larger rodent strain relative to commercially available mouse models.

Materials and Methods

Orthotopic glioblastoma and metastatic breast cancer PDX: Briefly, patient-derived tumor cells isolated from either glioblastoma or metastatic breast cancer located in the brain were transduced to express tdTomato-luciferase. 6 SRG rats were implanted with $1x10^6$ T9 cells in 10 µL PBS. Orthotopic glioblastoma CDX: Three SRG rats (CRL strain #707; female) each were inoculated with $5x10^5$ luciferase expressing human glioblastoma cells in 5-10 µL PBS, U87MG-luc and U118MG-luc to establish xenograft tumors in the striatum of the rat brain. Diffuse intrinsic pontine glioma (DIPG) PDX: Briefly, patient-derived DIPG cells (denoted DIPGXIX) were transduced to express tdTomato-luciferase before implantation. One SRG rat was implanted with $1x10^6$ DIPGXIX cells in 10 µL PBS. All injection coordinates, relative to bregma: +1.2mm anterior, 3mm lateral left, -6.5mm dorsoventral. Tumor growth for all conditions was monitored via in-life bioluminescent imaging after subcutaneous injection of D-luciferin (150 mg/kg).

Orthotopic Glioblastoma PDX Model

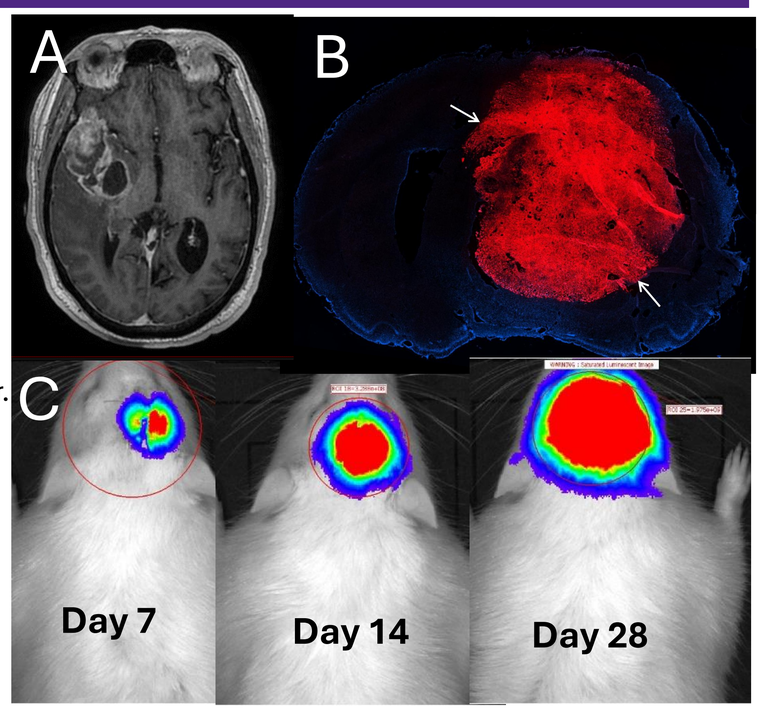


Figure 1 (Right): Orthotopic patient-derived xenograft in a new glioblastoma (GBM) SRG rat model. A) Gadolinium-enhanced axial preoperative brain MRI from a patient just prior to GBM resection B) GBM cells isolated from a patient tumor were genetically engineered to express firefly luciferase for bioluminescence imaging (BLI) and the tdTomato red fluorescent reporter. This brain section was treated with DAPI stain (blue) and houses a tumor 28 days post-implantation of GBM cells (red, arrows) obtained from the patient tumor shown in the MRI. C) Representative BLI monitoring of orthotopic human GBM growth in the SRG rats.

Results: DIPG

Diffuse midline glioma, colloquially referred to as Diffuse Intrinsic Pontine Glioma (DIPG), is a high-grade brainstem cancer, which remains the leading cause of brain-tumor related deaths in children. Radiation is standard of care for DIPG; however, this can only provide palliative symptomatic relief and it is estimated that only 10% of afflicted individuals survive beyond 2 years.

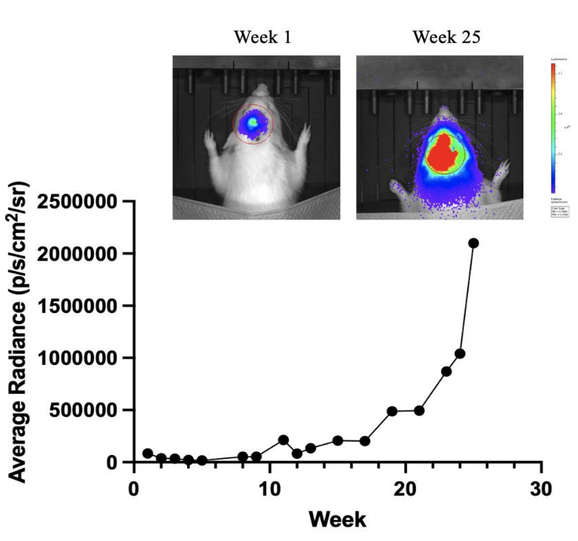


Figure 4 (Right): Orthotopic patient-derived xenograft in a new diffuse intrinsic pontine glioma (DIPG) SRG rat model. A) Bioluminescent images for n=1 SRG rat implanted with DIPGXIX PDX ($1x10^6$ cells). Graph depicts the peak radiance values (p/s/cm²/sr) over time.

Conclusions

- SRG rats support intracranial tumor establishment and growth from cell line derived and patient derived sources, and of glial and breast origin
- The increased size of the SRG allows for larger tumor burden before endpoint
- Novel therapeutics can be delivered via oral, intravenous, intraperitoneal, or intracranial delivery through bolus injection or implanted pump
- Permissive to serial blood collection for pharmacokinetic assessment within a therapeutic efficacy study

Results: Orthotopic Glioblastoma CDX

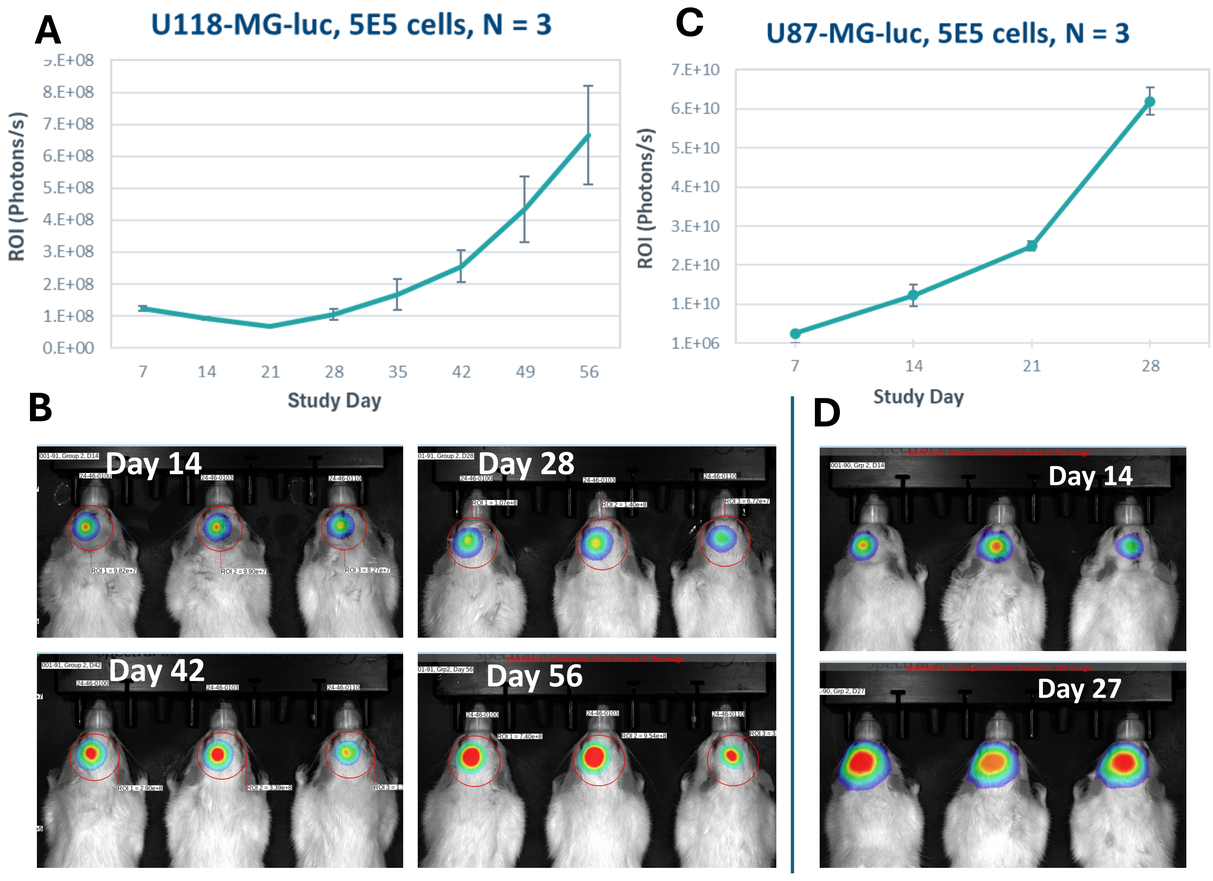
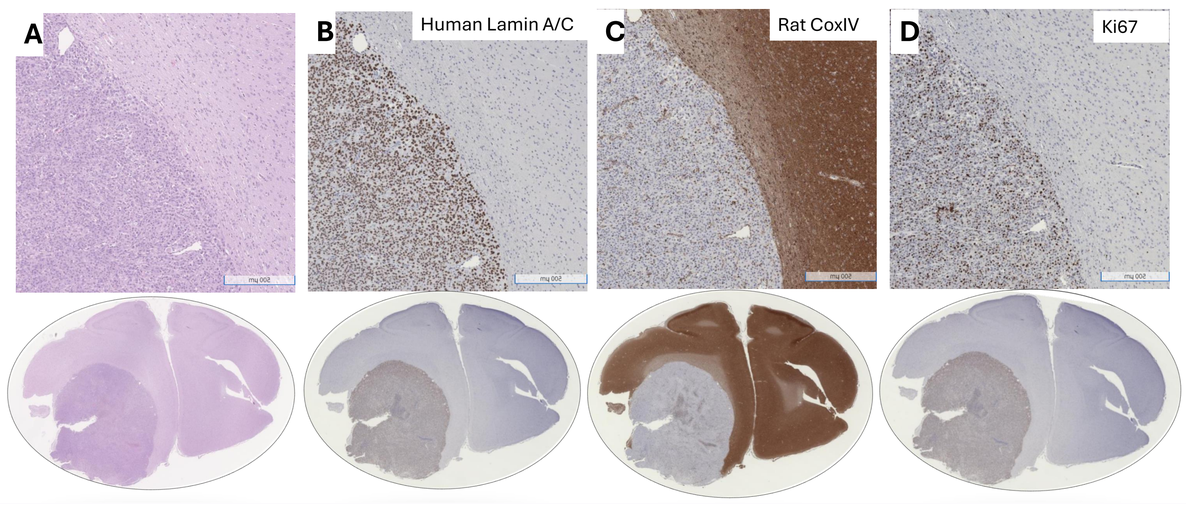


Figure 2 (Above): Cell derived xenograft (CDX) orthotopic glioblastoma models in the SRG Rat. A-B) U118-MG-luc, and C-D) U87-MG-luc cells were inoculated into the striatum of 3 female SRG rats each. Post-implant day and BLI values are shown.

Figure 3 (Below): Representative immunohistochemistry staining of U87-MG-luc CDX tumors. A) H&E, B) Human Lamin A/C, C) Rat CoxIV, and D) Ki67.



Results: Metastatic Breast Cancer PDX

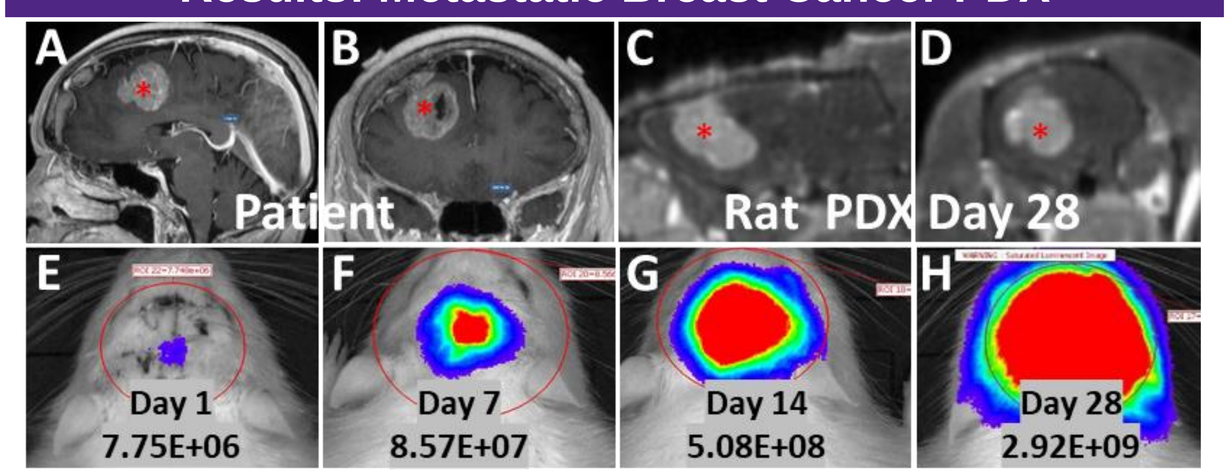


Figure 5 (Above): New breast cancer brain metastasis (BCBM) model in the SRG rat. A) Sagittal and B) coronal gadolinium-enhanced brain MRI scan in a human patient prior to BCBM resection. Patient tumor cells were isolated from operative tissue and subsequently implanted into SRG rat brains to initiate growth of large tumors, shown here with C) sagittal and D) coronal MRI. The patient and SRG tumors (asterisks) both elicited marked neovascularization, gadolinium uptake, and cerebral edema. E-H) Bioluminescence imaging (BLI) used to track human BCBM growth in the SRG rat brain. Post-implant day and BLI values are shown.

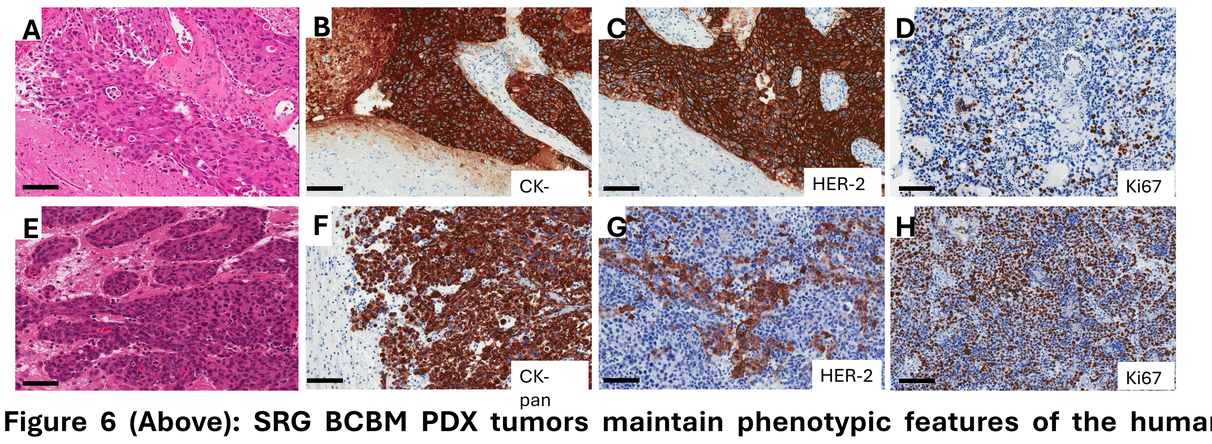


Figure 6 (Above): SRG BCBM PDX tumors maintain phenotypic features of the human patient tumor. (A-D) The original patient BCBM histology and representative immunohistochemistry staining. A) The BCBM, stained with H&E, is well demarcated from brain tissue, composed of moderated differentiated carcinoma with poorly formed glandular structures. B) It is strongly positive for pan-cytokeratin (CK), C) HER-2 and D) Ki67. (E-H) The PDX tumors created from patient BCBM cells are similarly well demarcated from the SRG rat brain. E) The PDX tumors created from patient BCBM cells are similarly well demarcated from the SRG rat brain (H&E stain). The carcinoma cells are poorly differentiated, as sheets or nests of epithelioid cells with no recognizable glandular formation. They are positive for H) pan-CK, G) HER-2 and H) Ki67.