In vivo subcutaneous and orthotopic cancer xenograft modeling in the Sprague-Dawley Rag2 null Il2rgamma null SRG rat (*OncoRat®*)

#42

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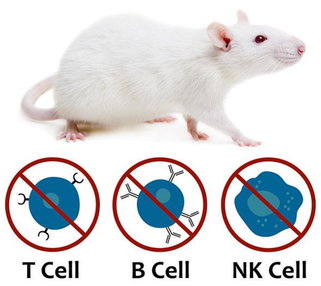
The SRG™ Rat

The Sprague Dawley *Rag2* null, *Il2rgamma* null ***SRG™*** rat lacks mature B, T, and circulating NK cells, rendering it the most immunodeficient rat available. The SRG rat readily supports growth of a variety of human tissue. Severe immunodeficiency in a larger size animal enables:

- Ability to obtain serial tumor biopsies and blood draws
- PK studies within efficacy studies.
- Greater ease of orthotopic surgical inoculations.
- Larger tumor size (up to 10x larger than mice) increases sample analysis options post-mortem.



Here we demonstrate the utility of the SRG rat for both subcutaneous and orthotopic xenograft modeling. The SRG rat supports the growth of both brain and liver orthotopic cancers. We use *in vivo* imaging to visualize tumor establishment and growth in orthotopic and metastatic models. These data confirm the SRG rat is an excellent host for modeling human diseases.

Subcutaneous Xenograft Models

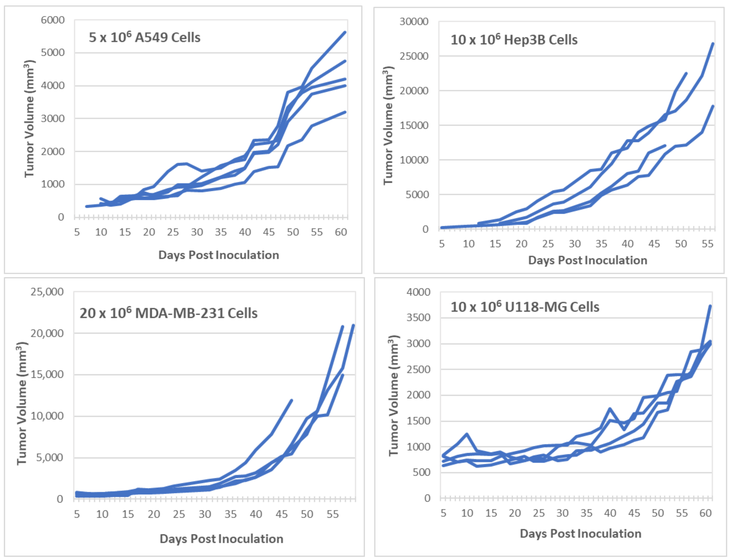
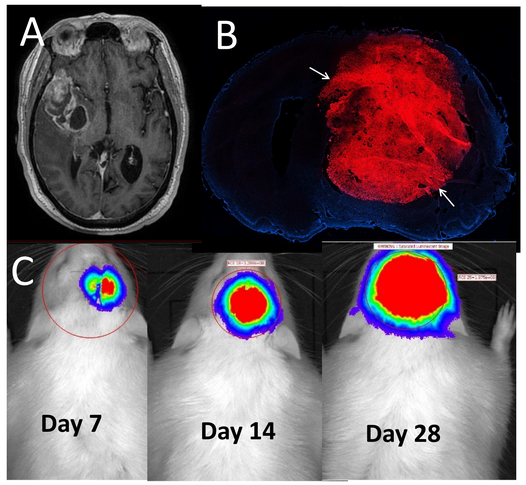


Figure 1 (Above): The SRG hosts subcutaneous xenograft tumors of various human origins with minimal variability and consistent growth kinetics. (A) A549 (ATCC CCL-185™) lung epithelial cell carcinoma (5x10^6), (B) Hep3B (ATCC HB-8064™) Hepatocellular carcinoma cells (10x10^6), (C) MDA-MB-231 (ATCC HTB-26™) triple negative breast epithelial carcinoma cells (20x10^6) and (D) U118-MG (ATCC HTB-15 ™) glioblastoma cells (10x10^6) were inoculated subcutaneously into the hind flank of SRG rats (n=4-5). Cells were suspended in 250µL PBS and inoculated in equal volume of VitroGel (TheWell Biosciences, VHM01).

Orthotopic Glioblastoma PDX Model

Figure 2 (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.



Orthotopic Liver Cancer Model

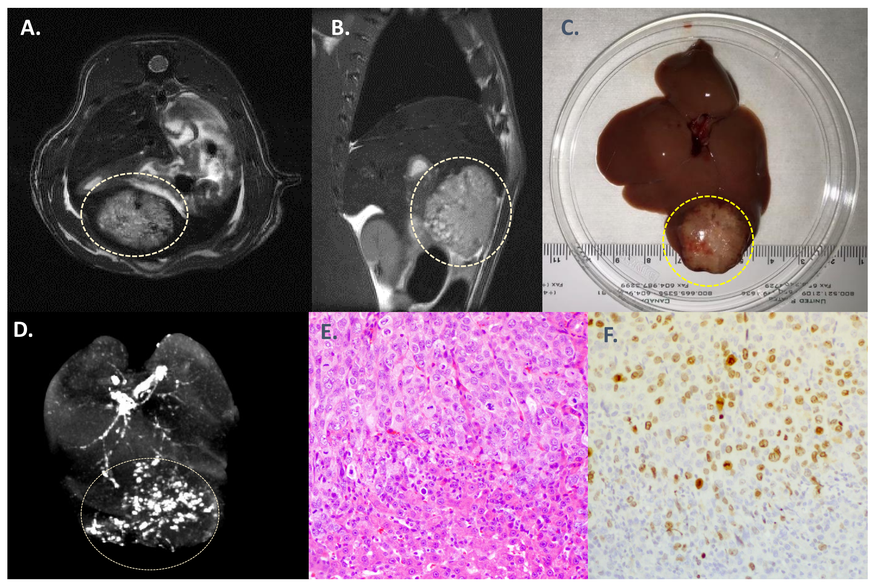


Figure 5 (Above): Hep3B (ATCC HB-8064™) Hepatocellular carcinoma cells (5x10^6) were directly inoculated into the left lateral lobe of the liver in SRG rats. At 37 days post inoculation, noninvasive MRI (Agilent [Varian] 7.0 T MRI) was performed to assess intrahepatic tumor growth. (A and B) Representative T2-weighted axial and sagittal MRI of tumor bearing rat. Tumor tissue appears hyperintense on T2-weighted image. (C) Photograph of excised liver and tumor. (D) Ex vivo computerized tomography (CT) image of excised liver/tumor following arterial barium perfusion demonstrating preferential hepatic arterialization of orthotopic Hep3B tumor. (E) Hematoxylin and eosin histological section demonstrating tumor and surrounding liver histology. Tumor tissue is located at the top of the micrograph. (F) Ki67 staining demonstrating enhanced proliferation of tumor cells. Tumor tissue is located at the top of the micrograph. Dotted circle indicates tumor.

Orthotopic Breast Cancer Brain Metastasis PDX Model

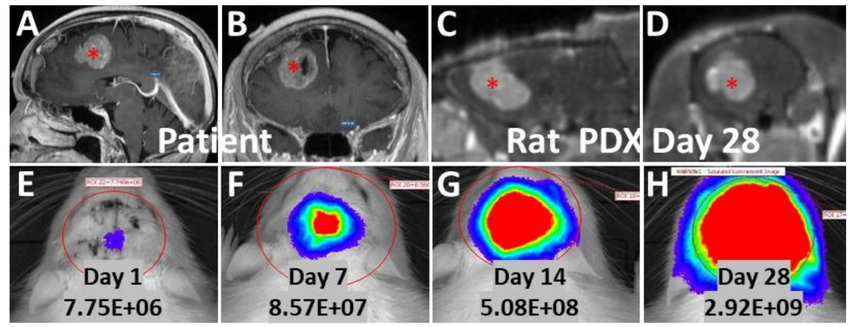


Figure 3 (Above): New breast cancer brain metastasis (BCBM) model in the SRG rat. A) Sagittal and B) coronal gadolinium-enhanced brain MRI scan in a human patient prior to BCBM resection. Patient tumor cells were isolated from operative tissue and subsequently implanted into SRG rat brains to initiate 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.

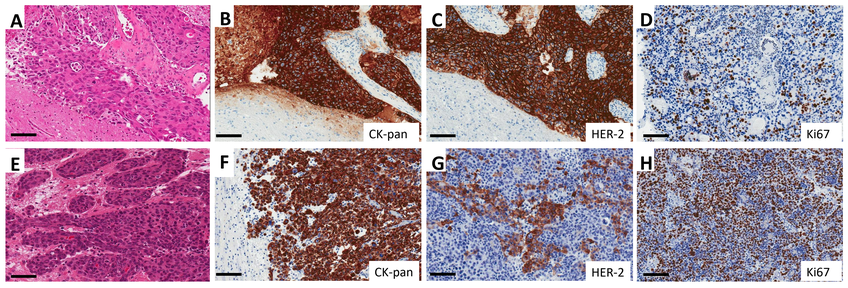


Figure 4 (Above): SRG BCBM PDX tumors maintain phenotypic features of the human patient tumor. (A-D) The original patient BCBM histology and representative immunohistochemistry staining. A) The BCBM, stained with H&E, is well demarcated from brain tissue, composed of moderated differentiated carcinoma 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.

Conclusions & Future Research

Conclusions

- SRG rats support orthotopic liver xenograft growth, detectable by MRI, CT, and histology.
- The SRG rat is an excellent model for brain metastasis. Breast cancer metastasis produces large PDX tumors hosted by the SRG rat and replicate the human tumor's histology and characteristics.
- SRG rats readily support a heavy tumor burden after intracranial inoculation of glioblastoma cells before onset of clinical signs.

Future Research

- Continued characterization of validated tumor xenograft models, both orthotopic and subcutaneous.
- Hera BioLabs will continue to characterize the tumor microenvironment in SRG rats compared to immunocompromised mice.