In vivo imaging of ovarian and non-small cell lung cancer models hosted in the Sprague-Dawley Rag2 null Il2rgamma null SRG rat (*OncoRat®*)

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Abstract

Human cancer xenografts are a vital tool for understanding tumor biology, growth kinetics, and therapeutic efficacy using animal models. While *in vivo* studies are traditionally done in immunocompromised mice, we have created the ***SRG™ OncoRat®)*** that is an excellent host for human xenografts. *In vivo* luminescent imaging has routinely been used in mouse models, and is particularly useful for studying orthotopic xenografts or metastatic models. In this study, we validated the SRG rat as a model that can effectively be used for *in vivo* imaging of human cancers.

For the current study, we assessed two tumor models. Ovarian cancer cell line OV81.2-luc is a luciferase positive cell line that was generated from ascites collection from a grade IIIC, serous ovarian carcinoma. Tumors were established by inoculating OV81.2-luc cells IP into female SRG rats and female NSG mice, then treated with vehicle or cisplatin for 28 days. Human non-small cell lung cancer line H358-luc is a luciferase positive and metastasizes to lung when injected subcutaneously into the hind flank of SRG rats. Tumors were measured thrice weekly with calipers. For both studies, tumors were measured via weekly *in vivo* luciferase imaging using Spectral Instruments AMI HT system.

Tumors established rapidly for both OV81.2-luc and H358-luc in both SRG rats and NSG mice, with luciferin positive signal starting one week post inoculation. In OV81.2-luc hosting animals, upon necropsy, tumors were found on multiple abdominal organs including the peritoneum, ovary, mesentery, intestines, kidneys, liver, and body cavity wall. Metastatic events outside the abdominal cavity were not evident in OV81.2-luc hosting animals. In H358-luc tumor bearing rats, a high metastatic tumor burden was found in the lungs.

These data confirm that the SRG rat is an excellent host for studying human cancer when compared to commonly used immunodeficient mouse models. Data demonstrate that the SRG rat has a high utility for studies using both *in vivo* imaging, such as orthotopic tumor implantation, and studies on metastasis. As the most immunodeficient rat commercially available, the SRG rat retains the ability to establish human tumors while possessing size, physiology, and metabolism-based advantages when compared to mice.

The SRG™ Rat

With a unique need for larger animals that support human tumor growth, the SRG continues to distinguish itself from immunocompromised mice.

- Sprague Dawley *Rag2* null, *Il2rgamma* null ***SRG™***.
- Lacks mature B, T, and circulating NK cells, rendering it the most immunodeficient rat available.
- Higher take rates with difficult cell lines such as VCaP
- Faster tumor growth with decreased variance observed with many cell lines
- The larger size of the rat enables serial tumor biopsies and blood draws
- Option to perform PK studies within efficacy studies
- Greater ease of orthotopic surgical inoculations

Materials and Methods

Establishment of human non-small cell lung cancer xenograft tumors: H358-luc tumors were inoculated subcutaneously into SRG rats (4 million cells) and NSG mice (1 million cells). Cells were mixed with Matrigel® 1:1 and injected subcutaneously in the hind flank. Tumors were measured three times weekly and recorded in StudyLog. Animals were euthanized before the tumors reached humane endpoints.

Establishment of human ovarian cancer tumors: Patient ascites-derived, high grade serous ovarian cancer cell line OV81.2-luc was inoculated IP into SRG rats and NSG mice (1 million cells each). 3 days post-inoculation, animals were imaged and randomized into treatment groups by maximum flux (photons/s). Rats and mice were dosed at 10 mL/kg with either Vehicle 1 (daily/QD gavage; 10% DMSO in 10% hydroxypropyl-β-cyclodextrin in PBS pH7.4), Vehicle 2 (twice weekly/BIW intraperitoneal injection; PBS pH7.4), Olaparib (Rats: 15 mg/kg. Mice: 50 mg/kg. Vehicle 1, QD gavage), or Cisplatin (Rats: 1 mg/kg. Mice: 5 mg/kg. Vehicle 2, BIW IP injection).

In vivo imaging: All animals were subjected to weekly bioluminescent *in vivo* imaging using Spectral Instruments Ami HT. 15 minutes prior to imaging, animals were injected with 150 mg/kg D-luciferin intraperitoneally (Perkin Elmer Xenolight).

Results: H358-luc

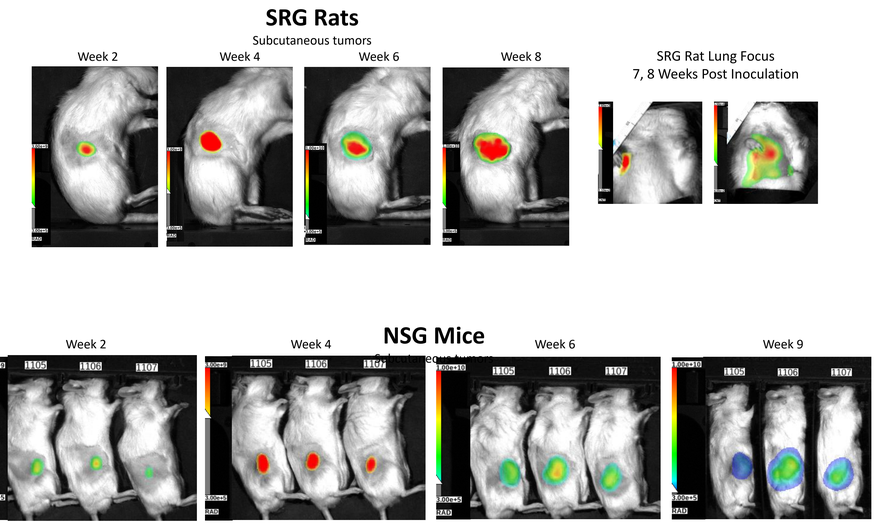

Figure 1: H358-luc Human Non-Small Cell Lung Cancer Xenografts in SRG Rats and NSG Mice. Subcutaneous tumor growth and lung metastases are shown for SRG rat (above) and NSG mice (below).

Conclusions & Future Research

Conclusions

The SRG rat provides numerous advantages over mouse models due to its size, providing enhanced tumor growth and ease of inoculations. In spite of its larger size, it is still small enough for in vivo bioluminescent imaging of deep tissues such as lung and peritoneum.

Future Research

Hera BioLabs will continue to characterize the tumor microenvironment in SRG rats compared to immunocompromised mice.

Results: OV81.2-luc

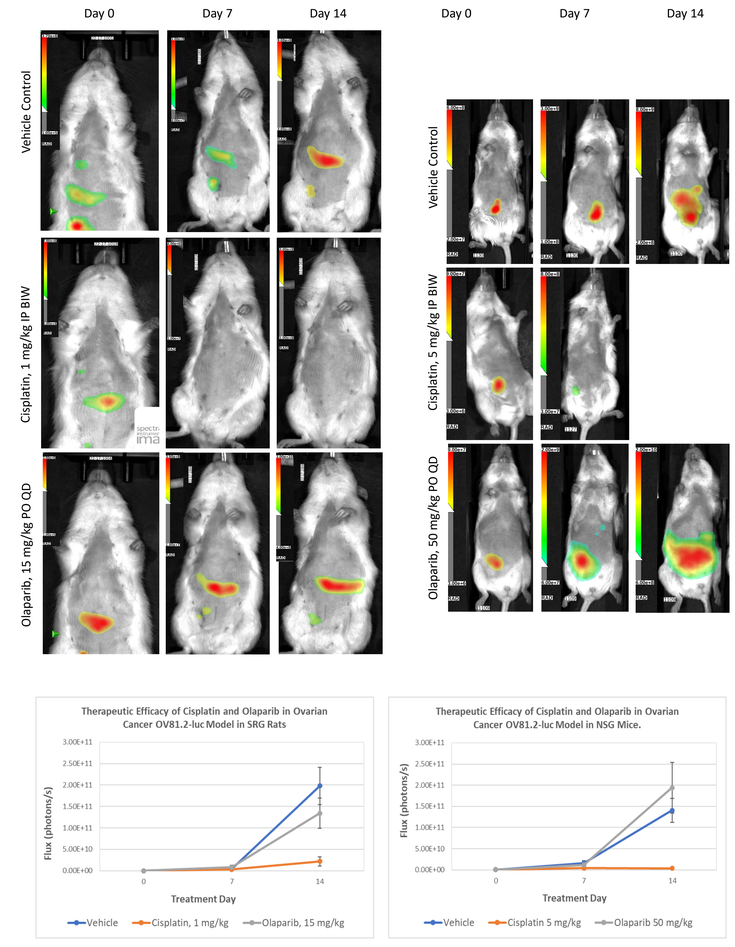

Figure 2: Orthotopic Ovarian Cancer modeling in SRG Rats (left) and NSG Mice (right). Representative images of 1million OV81.2-luc cells injected intraperitoneally in SRG rats (left) and NSG mice (right) treated with vehicle control, Cisplatin, or Olaparib.

Acknowledgements

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Reference

1. Noto, et al., 2020. The SRG rat, a Sprague-Dawley Rag2/Il2rg double-knockout validated for human tumor oncology studies. PL0S ONE 15(10): e0240169.