The *SRG*™ rat: A novel SCID rat for humanization studies

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Abstract

In vivo modeling of human cancer in genetically engineered rodents can provide insights into tumor kinetics, genetics and molecular biology, and allow for the testing of drug efficacy. Over recent years, studies have demonstrated that immunodeficient mice, such as the *NSG*™, reconstituted with functional human immune cells, such as peripheral blood mononuclear cells (PBMCs), are promising models for immuno-oncology efficacy studies. Immune humanized mice engrafted with human cancer cells show human-specific immune responses when treated with drugs that target immune pathways, such as CTLA-4 and PD-1, leading to the inhibition of tumor growth. These models provide a critical platform to study how the immune system can be engaged to drive anti-cancer efficacy.

Although the *NSG* and similar immunodeficient mouse strains have been beneficial for human immuno-oncology studies, there are many caveats to performing these studies in mice, including inconsistent tumor kinetics, small tumor size for downstream analyses, limited blood for pharmacokinetic (PK)/pharmacodynamic (PD) studies due to the small size of the mouse, and graft vs. host disease (GvHD) onset around 4-6 weeks post-engraftment. Humanized rat models would allow for the development of larger tumors and the ability to perform serial blood sampling on a routine basis throughout the course of treatment. We have created a Rag2 null, Il2rg null rat on the Sprague Dawley background (*SRG*™) that lacks B, T, and NK cells and supports the growth of multiple human cancer cell lines, including lines that do not engraft or grow well in existing mouse models such as the H358, and VCaP cell lines. The *SRG* rat is also permissive to immune humanization with PBMCs. PBMC-engrafted *SRG* rats have a significant amount of human CD45+ lymphocytes in peripheral blood, of which the majority are T cells, comparable to immune-humanized *NSG* and NOG mice. Some animals also show significant levels of circulating human B cells. Interestingly, the incidence of GvHD is delayed to 12-14 weeks post-engraftment in the immune humanized *SRG* rat. We have also assessed the ability of the *SRG* rat to support the growth of patient derived xenografts (PDX).

The generation of this novel humanized *SRG* rat model could allow for a more permissive host system to test existing and novel immunomodulatory strategies for the treatment of human disease.

Materials and Methods

Transplantation of human cancer cell lines: The specified number of cells for each cell line were mixed with Geltrex® or Cultrex® 1:1 and transplanted subcutaneously in the hind flank. VCaP prostate cancer cells – 10x106. Tumors were measured three times weekly and recorded in StudyLog to determine tumor growth kinetics. Animals were euthanized when the tumors reached humane endpoints. Serum PSA was measured by ELISA (ALPCO) on blood collected weekly after inoculation.

Transplantation of PDX tissues: Non-small cell lung cancer PDX tumor fragments were obtained fresh from patients at the Markey Cancer Center through the University of Kentucky Biospecimen Procurement and Translational Pathology Shared Resource Facility under IRB 17-0513-P3K. Each tissue was cut into 2mm x 2mm pieces and immediately implanted subcutaneously using a trocar into SRG rats or NSG mice. For serial transplants, tumors were removed aseptically and cut into 2mm x 2mm pieces and transplanted, flash frozen, or fixed in 10% NBF.

Immune humanization with PBMCs: Human PBMCs were purchased from Stem Express. 50x106 viable cells as determined flow cytometry analysis using propidium iodide were transplanted via the tail vein in *SRG* rats at 8-10 weeks of age. FACS analysis was performed to assess circulating human CD45+, human CD3+, human CD4+, human CD8+ and human CD20+ cells.

Results

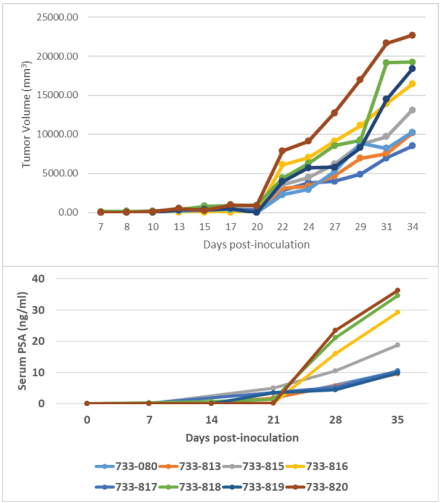

Figure1. VCaP tumor kinetics in the *SRG* rat. VCaP cancer cells, known to have poor engraftment rates and kinetics in immunodeficient mice, thrived in the *SRG* rat. The cells were inoculated in the flank region at a density of 10x106 cells per rat in a 1:1 solution of cell culture media and 5mg/ml Cultrex. Engraftment rate was 75%.

Top: Tumor growth. Each line represents growth in a single animal.

Bottom: Serum PSA. Blood was collected prior to inoculation and then weekly after inoculation. Serum was separated and assessed for PSA. Serum PSA is highly correlative with VCaP tumor growth in the *SRG* rat.

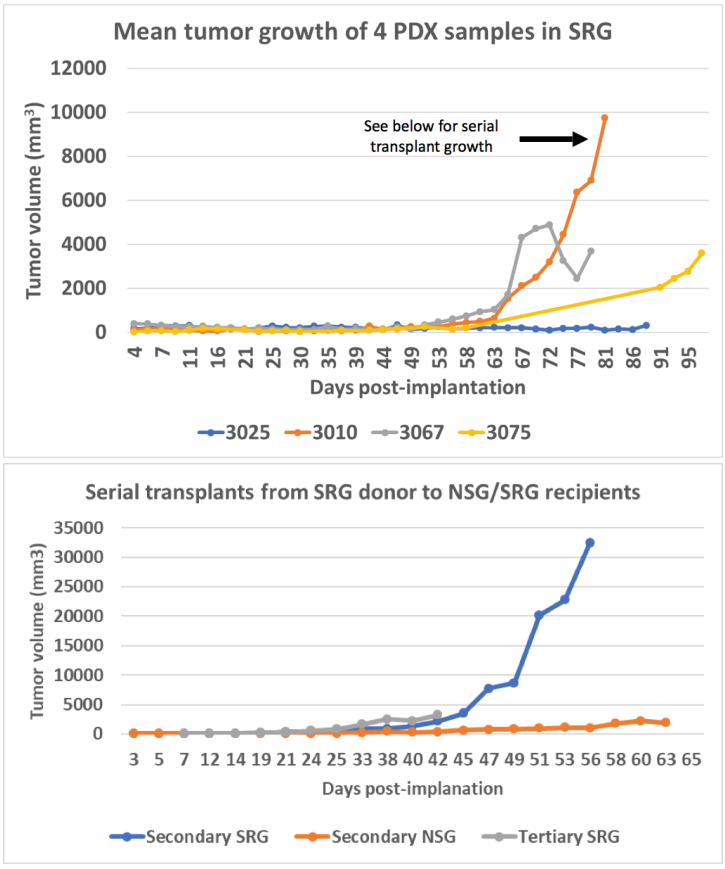

Figure 2. PDX tumor kinetics in *SRG*. Fresh NSCLC tumor samples were obtained and immediately implanted subcutaneously into 3 SRG rats each. **Top:** Primary transplants. Each line represents average tumor kinetics for 4 different patient samples. **Bottom:** PDX tumor sample from a primary growth (sample 3010, above) was harvested from one SRG and serially transplanted into 3 *SRG* (blue line) and 3 *NSG* mice (orange line). The secondary transplanted resulted in growth, which was then harvested from one *SRG* and serially transplanted again (tertiary) into 3 *SRG* rats (grey line).

	1°			2°		3°	
Sample	Recipients	Take rate	# pieces cryopreserved	Recipients	Take rate	Recipients	Take rate
3023	3 SRG	33%	51	N/A	N/A	N/A	N/A
3010	3 SRG	100%	215	2 SRG 3 NSG	100% 100%	3 SRG N/A	100% N/A
3067	3 SRG	67%	120	4 NSG	100%	3 NSG	100%
3075	3 SRG	33%	103	3 SRG 3 NSG	67% 67%	N/A	N/A
3095	3 SRG	33%	69	3 SRG 3 NSG	100% 100%	3 SRG N/A	100% N/A
3110	1 SRG	0%	0	N/A	N/A	N/A	N/A

Figure 3. Percent efficiency of engraftment for serially transplanted PDX tissues. NSCLC PDX samples were collected from 6 patients and transplanted into 3 *SRG* each (3110 was implanted into 2 SDR rats and 1 *SRG*). Those that grew were serially transplanted 1-2 more passages in the indicated species, with their respective percent engraftment shown. **Typical mouse tumor of ~2000mm3 yields 15-20 pieces for serial transplant. A single rat tumor >10,000mm3 yields 80-100 pieces.**

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Figure 4: Immune humanization of the *SRG* rat with human PBMCs

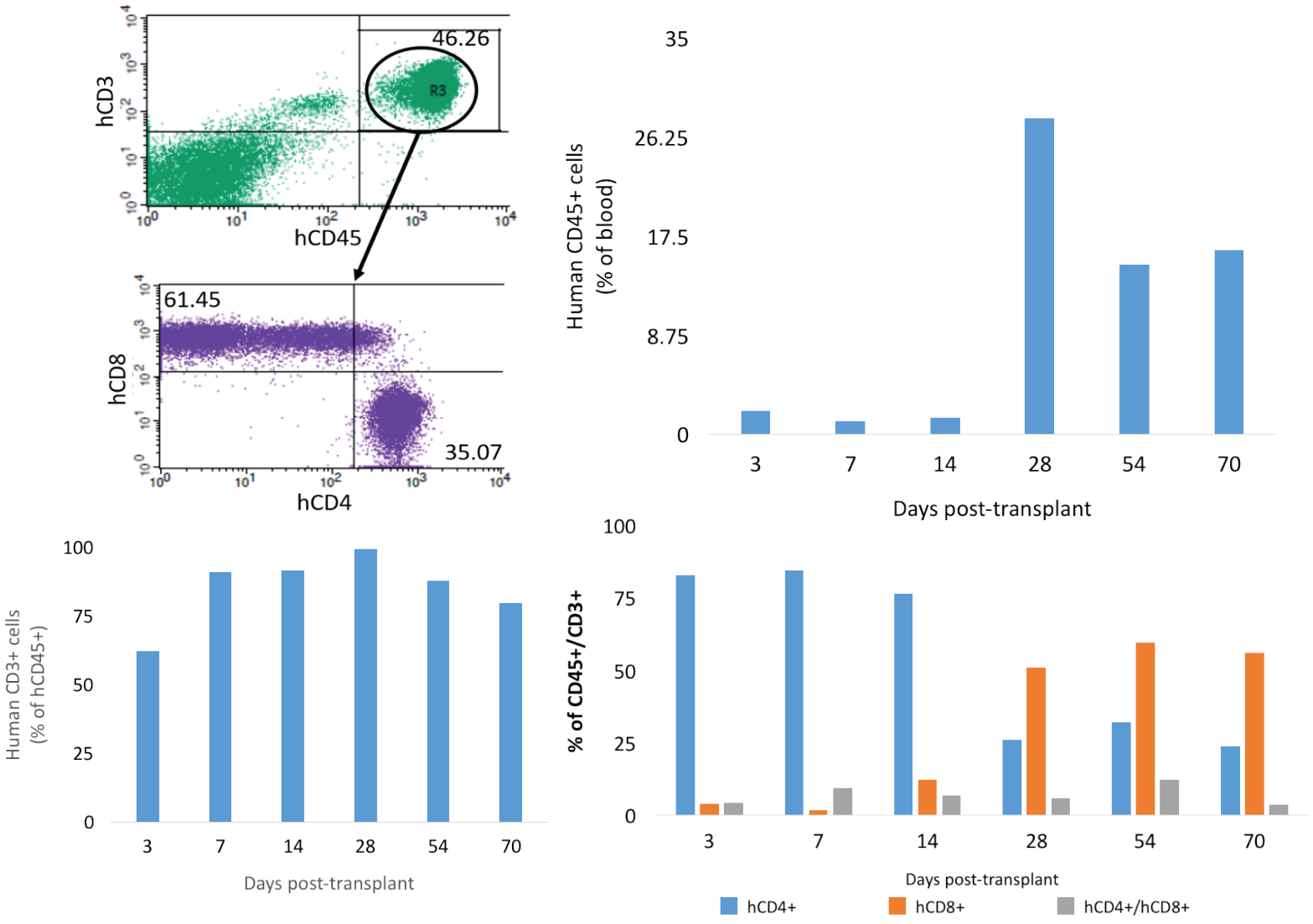

Figure 4: Immune humanization of the *SRG* rat with human PBMCs. Cryopreserved human PBMCs were purchased from Stem Express. Viability was determined by staining the cell suspension with propidium iodide and analyzing on a flow cytometer. 50x10^6 viable human PBMCs in 500µl PBS were injected into the tail vein of 3 male and 3 female *SRG* rats at 8-10 weeks of age. Peripheral blood was analyzed for the presence of human CD45+, CD3+, CD4+, and CD8+ cells at 3, 7, 14, 28, 54, and 70 days post-injection. By 4 weeks post-transplant, recipients had an average of 29% circulating human CD45+ cells. As of 10 weeks post-transplant, recipients have up to 46% human CD45+ cells and remain healthy. This study is currently ongoing to assess longevity of the human cells and the incidence of graft vs. host disease.

Conclusions

1. The *SRG* rat models support the growth of VCaP cancer cells, which are typically difficult to grow in mouse models.
2. Serum PSA levels are highly correlative with VCaP tumor growth in the *SRG* rat.
3. The *SRG* rat supports the growth of PDX samples, and these PDX samples can be serially transplanted from *SRG* to *SRG* and from *SRG* to *NSG* with high engraftment rates in serial passages.
4. The *SRG* rat is permissive to immune humanization with PBMCs, which could provide a model for immuno-oncology efficacy studies.

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