Novel immunodeficient rat models capable of supporting the growth of human tumor xenografts

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Abstract

Mouse models of human cancer have paved the way for studying cancer biology and genomics and their effects on cancer growth kinetics, propensity for metastasis, and treatment response. In addition, human cancer xenografts provide the opportunity to study cancer cell interactions with host stroma and tumor morphology. A plethora of genetically immunodeficient mouse models exist with different immune phenotypes, resulting in significant variability in tumor take rates and growth kinetics for a wide range of human cancer cell lines and patient derived xenografts (PDX). Inconsistent or poor growth in these immunodeficient models have made downstream analysis and drug efficacy testing difficult. As a result, a significant number of mice are needed for drug efficacy screening to achieve a cohort of animals with tumors of similar size with similar tumor growth kinetics for treatment. It is possible that these cell lines might grow more consistently in a different immunodeficient model, such as an immunodeficient rat.

Until recently, the only immunodeficient rat that existed is the nude (NIH-*Foxn1^{rnu}*; RNU) rat. This rat lacks T cells, but maintains a normal repertoire of B and NK cells. As such, there are a limited number of human cancer cell lines that can survive in the nude rat. We have created a genetically modified rat with a functional mutation of the Rag2 gene (Sprague Dawley – *Rag2* null; SDR), resulting in a loss of mature B and T cells. In addition, we have created a *Rag2/Il2rg* double knockout rat (Sprague Dawley – *Rag2; Il2rg* null; SRG) that lacks mature B cells, T cells, and has fewer NK cells than wild-type Sprague Dawley rats. We have shown that the SDR rat is permissive for solid tumor growth of the human acute lymphocytic leukemia REH cell line, human glioblastoma U87MG cell line, human non-small cell lung cancer H358 cell line, and cell lines derived from ovarian and endometrial PDX samples. In some cases, tumor growth kinetics are superior in the SDR rat compared with immunodeficient mouse models. We have demonstrated that HCT116 cells and the human prostate cancer cell line, VCaP, which has poor engraftment efficiency and growth kinetics in the mouse, grows well in the SRG rat. The SRG rat is currently being validated for growth kinetics of other human cancer cell lines and PDX tissues. In addition, we are developing several disseminated human leukemia models in the SRG rat and creating immune humanized mice and rats to be used in conjunction with human tumor xenografts for immunotherapy efficacy studies.

Materials and Methods

Generation of SDR™ (Sprague Dawley-Rag2 KO) and SRG™ (Sprague Dawley-Rag2;Il2rg KO) rats: SDR™: the Rag2 locus was targeted using XTN™ technology in spermatogonial stem cells (SSCs). Pooled SSCs were transplanted into DAZL-deficient sterile males and mated with wild-type Sprague Dawley rats. DNA was isolated from offspring and a male with a 27bp deletion was detected. SRG™: the Rag2 and Il2rg loci were targeted using CRISPR via PNI.

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. H358 NSCLC KRAS mutant - 1, 5, or 10 million. VCaP prostate cancer cells – 10e6. HCT116 – 2.5e6 cells in both NSG mice and SRG rats. PDX-derived cancer cell lines – 2e6 or 5e6. Tumors were measured three times weekly and recorded in StudyLog to determine tumor growth kinetics. Animals were euthanized when the tumors reached humane endpoints.

Immune humanization with PBMCs: Human PBMCs were purchased from Stem Express. 50e6 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 at 3, 7, 14, and 28 days to assess circulating human CD45+, human CD3+, human CD4+, human CD8+ and human CD20+ cells.

Results

Figure 1: Enhanced survival of NSCLC cell line H358 and tumor kinetics in the SDR™ rat compared to mice

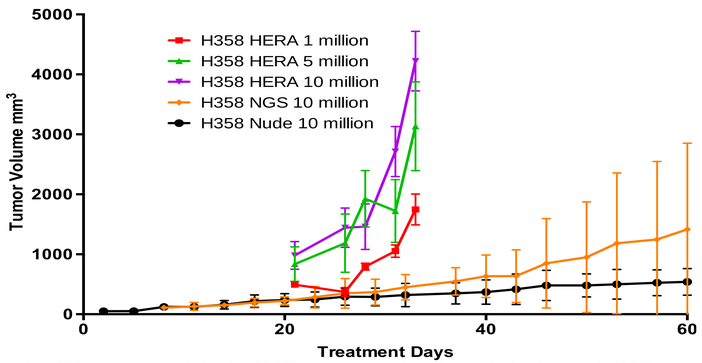

Figure 1. H358 tumor growth in the SDR™ rat compared to the nude (nu/nu) and NSG™ mice. H358 cancer cells were transplanted subcutaneously in the SDR™ rat. Three groups of 6 rats received either 1e6, 5e6, or 10e6 cells in 5mg/ml Geltrex®. Growth rate was directly proportional to the amount of cells transplanted. Take rate was 100%. These data are displayed in conjunction with data from the lab of Dr. Goutham Narla showing tumor growth kinetics of the H358 cell line in Nude and NSG™ mice, both of which were transplanted with 10e6 cells subcutaneously.

Figure 2. VCaP tumor growth in SRG™ rats.

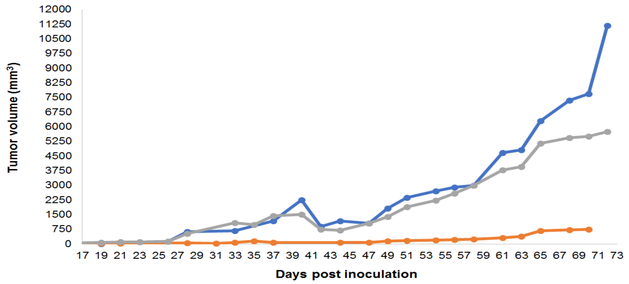

Figure 2. VCaP tumor growth in the SRG™ rat. VCaP cancer cells, a cell line with poor take rate and growth kinetics in immunodeficient mice, were transplanted subcutaneously in the SRG™ rat. Five male rats each received 10e6 cells in 5mg/ml Geltrex®. Take rate was 60%. **A follow-up study is in progress with a larger cohort of SRG™ male rats.**

Figure 3: Comparison of ovarian PDX-derived cell line growth kinetics in the NSG mouse vs. SRG™ rat

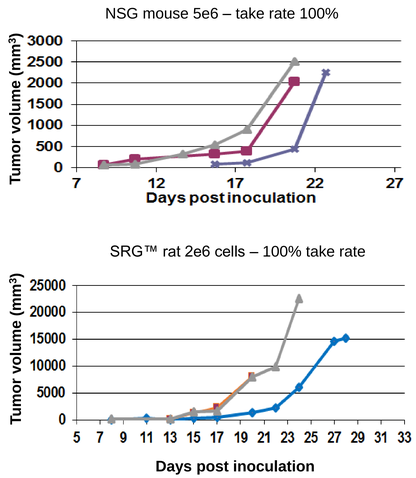

Figure 3. Ovarian PDX-derived cell line growth kinetics in NSG mouse vs. SRG™ rat. A cell line derived from an ovarian PDX tissue was transplanted SQ into NSG mice (5e6) and SRG™ rat (2e6). Take rate was 100% in both species (3 animals each). First measurable tumors appeared within 10 days for both species. Tumor growth rate is faster in the rat compared with the mouse.

Figure 4: Comparison of HCT116 growth kinetics in the NSG mouse vs. SRG™ rat

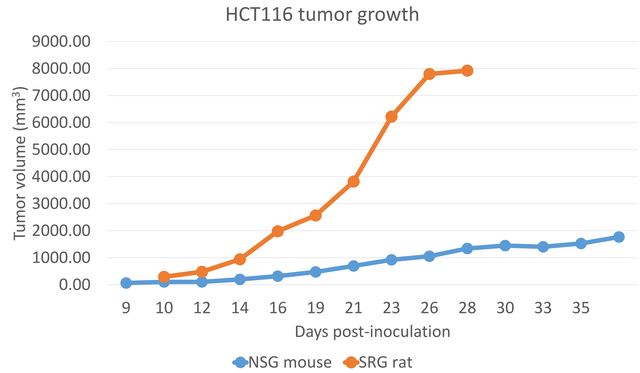

Figure 4. HCT116 tumor growth in the SRG™ rat compared to the NSG™ mouse. HCT116 cancer cells were transplanted subcutaneously in the SRG™ rat and NSG mouse. Six SRG™ rats and 5 NSG mice each received 2.5e6 cells in 5mg/ml Cultrex® BME 3. Take rate was 100% in both species. Tumor growth rate was significantly faster in the rat compared with the mouse.

Figure 5: Humanization of the SRG™ rat immune system with PBMCs

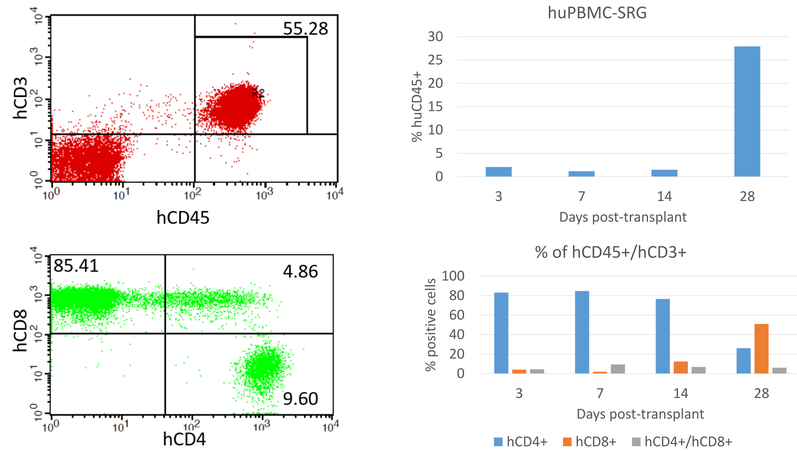

Figure 5: Immune humanization of the SRG™ rat with human PBMCs. Cryopreserved human PBMCs were purchased from Stem Express. Viability was determined by staining the cell suspension with propidium iodide and analyzing on a flow cytometer. 50e6 viable human PBMCs in 500µl PBS were injected into the tail 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+, CD8+, and CD20+ cells at 3, 7, 14, and 28 days post-injection. No B cells (CD20+) were detected (not shown). By 4 weeks post-transplant, recipients had up to 87% circulating human CD45+ cells (average 29%). This study is currently ongoing to assess longevity of the human cells and the incidence of graft vs. host disease.

Conclusions

1. Both the SDR™ and the SRG™ rat support the growth of various human cancer cell lines.
2. The NSCLC KRAS mutant cell line H358 has 100% survival when transplanted in the SDR™ rat and shows faster and more uniform growth kinetics compared to growth in the Nude and NSG™ mouse.
3. VCaP, a cell line that has poor take rate and growth kinetics in the mouse models, grows well in the SRG™ rat.
4. HCT116 cell line has a faster tumor growth rate in the SRG™ rat compared with the NSG™ mouse.
5. The SRG™ is permissive to immune humanization with PBMCs, which could provide a model for immuno-oncology efficacy studies.

Acknowledgements

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