A Novel Immunodeficient Rat for Modeling Human Cancer

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807

Abstract

Animal models of human cancer offer the potential to study human tumor growth kinetics, genetic variance among human cancers, and provide in vivo platforms for drug efficacy testing. In particular, immunodeficient mouse models have been invaluable in modeling a wide range of human cancers and testing drug efficacy. However, the use of mouse models is limited by the lack of growth of many cancer cell lines in mice, the variability of growth kinetics and take rates from mouse to mouse. Drug efficacy studies are difficult due to the limited number of cell line-based models to test novel agents, the large sample sizes needed to power mouse in vivo studies, and the small tumor size and lack of ability to perform serial sampling of tumor and blood for pharmacodynamic/pharmacokinetic studies. These challenges also occur in patient derived xenograft (PDX) models in which take rates are even lower and growth rates slower to obtain sufficient numbers of tumors in mice for drug efficacy studies. Mice are also limited in tumor growth potential with regard to humane endpoints and their small size also limits the volume of blood that can be collected for pharmacokinetic and toxicology analysis. An immunodeficient rat model could provide a solution to these issues by allowing for larger tumor size, easier surgical manipulation, and greater volume of tissue and blood sampling for downstream analyses. In addition, large tumors from rats could be serially transplanted into mice for drug efficacy testing and could provide a large number of transplanted mice in a shorter period of time compared with serially transplanting from mouse to mouse.

We have created an immunodeficient rat model with a functional deletion of the Rag2 gene. This knockout, created using spermatogonial stem cells, lacks mature B and T cells. To assess the capability of the Rag2 knockout rat to accept human xenografts, we transplanted commercially available human cancer cell lines into our animals. Here, we show tumor growth in the Rag2 knockout rat (SDR™) transplanted subcutaneously with the human glioblastoma cell line U87MG and the non-small cell lung cancer KRAS mutant cell line H358. Studies are underway to characterize the SDR™ rat's ability to grow other human cell lines, including those that do not grow well in mice, and PDX tissues. In addition, we are characterizing a Rag2; Il2rg double knockout rat (SRG™) for human cancer xenograft efficiency.

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.

FACS analysis of immune cells: To detect T, B, and NK cells, flow cytometric analysis was performed on splenocytes and thymocytes. Cells were stained with fluorophore-labeled antibodies at a final concentration of 25µg/ml in 20µl volume for 20 minutes. Antibodies used were Goat anti-rat IgM-APC (Stem Cell Technologies #10215), PE Mouse anti-rat IgM (BD #553888), FITC Mouse anti-rat CD45R (BD #561876), APC Mouse anti-rat CD45R (BD #554881), FITC Mouse Anti-Rat CD8b (BD #554973), PE Mouse anti-rat CD8b (BD #554857) APC Mouse Anti-Rat CD4 (BD #550057), FITC Mouse Anti-Rat CD161a (BD #561781), APC Mouse anti-rat CD161a (BD #555009).

Transplantation of human cancer cell lines: 1 million cells (U87MG human glioblastoma) or 1, 5, or 10 million cells (H358 human non-small cell lung cancer cells) were mixed with Geltrex® 1:1 and transplanted subcutaneously in the hind flank. Tumors were measured three times weekly and recorded in StudyLog to determine tumor growth kinetics. Animals were euthanized before the tumors reached humane endpoints.

Immunohistochemistry for human proteins: Tumors were excised and fixed in 10% NBF. Standard 5um sections were collected and human cells were visualized by staining with an antibody that recognizes a protein found in all human mitochondria (mouse anti-human mitochondria antibody, clone 113-1; EMD Millipore #1273) at 1:250.

Results

Figure 1: Immunophenotype of SDR™ rats

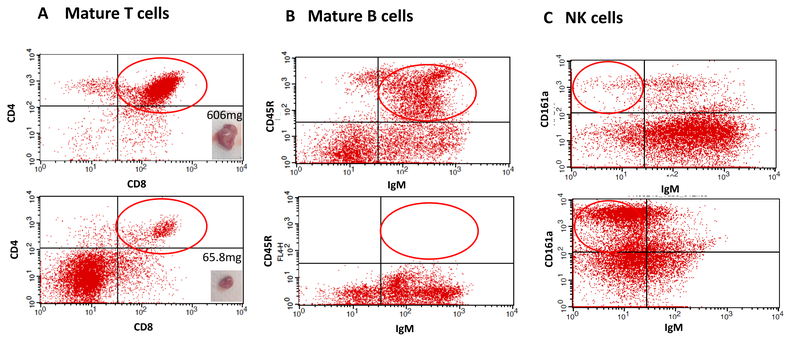

Figure 1. Analysis of immune cell populations in the SDR™ rat. A) SDR™ rat thymocytes contain fewer mature T cells (bottom panel), compared to a wild-type control (top panel). Insets show thymus with organ weight. The majority of thymocytes are CD4- and CD8-double negative in the SDR™ rat. B) The SDR™ spleen contains no mature B cells as demonstrated by lack of CD45R (B220)+/IgM+ cells (bottom panel) compared to wildtype spleen (top panel). C) SDR™ spleen has an increased NK cell population (bottom panel) compared to the wild-type (top panel).

Figure 2. U87MG tumor growth in SDR™ rats.

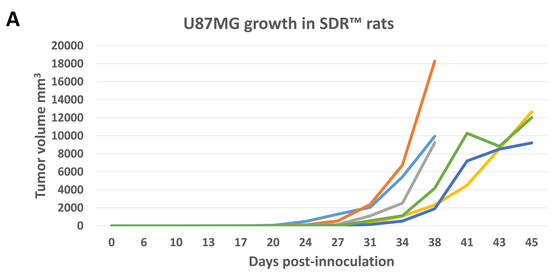

Figure 2. Subcutaneous growth of the human glioblastoma cell line U87MG. 1x10^6 U87MG cells resuspended in Geltrex® at 5mg/ml were injected subcutaneously into SDR™ rats. A) Graph showing tumor volume over time (mm^3). Each line represents an individual rat.
B) Tumor growth in an SDR™ rat.

Figure 3: Immunohistochemistry of tumors derived from human U87MG cells

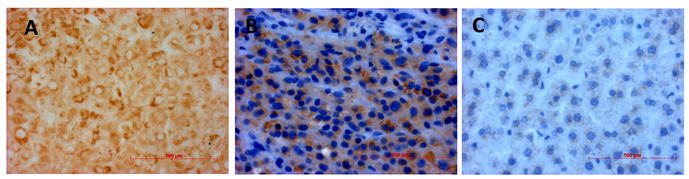

Figure 3. Immunohistochemistry for human-specific proteins in tumors derived from U87MG cancer cells. Immunohistochemistry with an antibody that specifically recognizes a protein in human mitochondria reacts with sections taken from the tumors. A) Brown staining of human mitochondria protein in a tumor from an animal transplanted with U87MG cells. Staining demonstrates peri-nuclear localization of the protein. B) Antibody staining with hematoxylin counterstain in serial section. C) The antibody for human mitochondria protein does not show staining in tissue from a rat that was not injected with human cells. 40x magnification. Scale bar = 100µm.

Figure 4: Enhanced survival of NSCLC cell line H358 and tumor kinetics in the SDR™ rat compared to mice

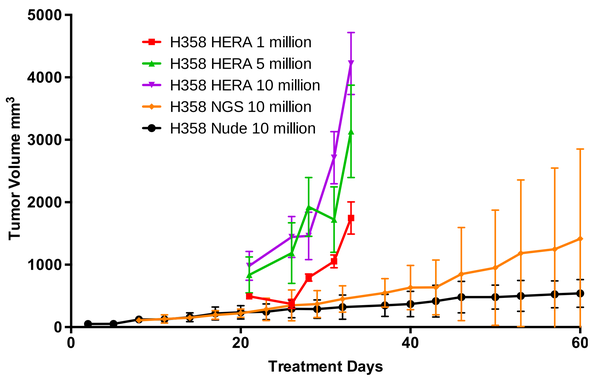

Figure 4. H358 cancer cells growth in the SDR™ rat compared to the nude (nu/nu) and NSG™ mice. H358 cancer cells were transplanted subcutaneously in the SDR™ rat. Three groups of 6 rats received either 1e6, 5e6, or 10e6 cells in 5mg/ml Geltrex®. Growth rate was directly proportional to the amount of cells transplanted. 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 5: Immunophenotype of SRG™ rats

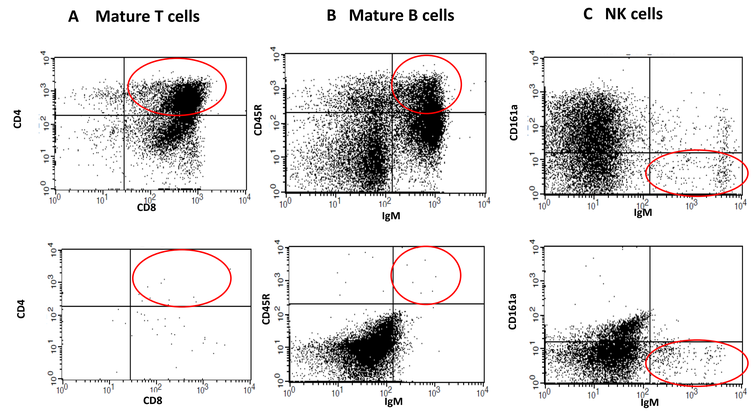

Figure 5: Analysis of immune populations in SRG™ rats. A) CD4+/CD8+ mature T cells are absent from SRG™ rat thymocytes (bottom panel), compared to a wild-type control (top panel). The lack of thymus tissue in the SRG™ rat results in a low recovery of thymocytes. B) The SRG™ spleen contains no mature B cells as demonstrated by lack of CD45R (B220)+/IgM+ cells (bottom panel), compared to WT spleen (top panel). C) The Il2rg knockout in the SRG™ rat results in a reduced NK cell population (bottom panel) compared to the SDR™ rat, which only has a Rag2 knockout (compare to figure 1, panel C). NK cells in the SRG™ rat are similar to or less than the amount of NK cells in the WT rat (top panel).

Conclusions

1. The SDR™ rat lacks mature T and B cells. The SRG™ rat lacks mature T and B cells, and also has a much lower proportion of NK cells compared to the SDR™ rat due to the knockout of Il2rg.
2. We have demonstrated that several commercially available human cancer cell lines grow well in the SDR™ rat.
3. 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.
4. Studies are underway to characterize human cancer xenografts in the SRG™ rat and human patient-derived xenografts (PDX) in the SDR™ and SRG™ rats.

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