Abstract

Immunodeficient mouse models seek to recapitulate the clinical features of advanced human cancers. However, drug efficacy testing and downstream analysis such as pharmacokinetic (PK) /pharmacodynamic (PD) based studies are limited because of variability in tumor growth and kinetics, limited tumor growth potential, requiring the implantment of larger numbers of mice in each treatment group to achieve required cohort sizes. Considering the distinctive advantages of rats in terms of their larger size, and ease of sampling at different time points for drug efficacy and PK/PD testing, we have developed unique immunodeficient rat models: the Rag2 knock out SDR™ rat (absence of mature B and T cells) and the Rag2/Il2g double knockout SDR™ rat (absence of mature B and T cells, and lower NK cell) on a Sprague-Dawley background. Our group has previously demonstrated better tumor kinetics, and increased volume of xenografts in these rat models compared to available immunodeficient mouse strains for various cancer xenograft models. The purpose of the present study is to demonstrate the feasibility of drug efficacy studies using targeted molecular agents directed against cancer- associated drivers in our newly developed SDR and SDR rat models. We tested the efficacy of a combination treatment of an AKT inhibitor (MK2206) and mTOR inhibitor (AZD6244) in a SDR rat xenograft model of human KRAS-mutant non-small cell lung cancer. Isotype SDR™ rats were transplanted subcutaneously on the left hind flank in SDR rats. When tumor size reached 100-150 mm³, the rats were randomized to either control or treatment group. The treatment group received a combination of AZD6244 with MK2206, and the vehicle only control received n, n-dimethylacetamide + SoluTab®/H3 Il VS 15, twice daily for 30 days by oral gavage. The kinase-inhibitor induced the growth of lung tumor xenograft in the SDR rats as shown by decreased mean tumor volume and fold changes in tumor volume compared to the control group. The combination treatment dose was just as effective at half the dose reported in previous mouse studies, demonstrating that lower dosing is sufficient for efficacy testing in rats. Importantly, no mortality, behavioral abnormalities, or changes in body weight were observed during the study indicating a favorable tolerability profile in this model.

We have also demonstrated that a human prostate cancer cell line VCaP which has very poor engraftment and growth profiles in existing mouse models, grows well in our SDR rats. The SDR rat is now being used to study the chemotherapeutic efficacy of the benchmark androgen-receptor inhibitor Enzalutamide, in this VCaP tumor xenograft model.

Materials and Methods

Generation of SDR™ (Sprague Dawley-Rag2 KO) and SDR® (Sprague Dawley-Rag2/Il2g KO) rats: SDR™: the Rag2 locus was targeted using CRISPR technology in oogonial stem cells (SSCs). Pregated SSCs were transplanted into SKG deficient sterile males and mated with wild-type Sprague Dawley rats. DNA was isolated from offspring and a male with a 2:10 deletion was selected. SDR®: the Rag2 locus and Il2g were targeted using CRISPR or PNA.

Transplantation of human cancer cell lines: The specified number of cells for each cell line were mixed with Certo® or Certo®/Rag2.1:1 and transplanted subcutaneously in the hind flank. H358 NSCLC KRAS mutant - 1, 5, or 10 million. VCaP prostate cancer cells - 100,000. Tumors were measured three times weekly and recorded in StatLog to determine tumor growth kinetics. Animals were euthanized when the tumors reached humane endpoints.

Immunohumanization with PBMCs: Human PBMCs were purchased from Stem Express. 506 viable cells as determined flow cytometry analysis using propidium iodide were transplanted via the tail vein in SDR rats at 8-10 weeks of age. FACS analysis was performed at 7, 14, 28, 56 and 70 days to assess circulating human CD4+, human CD3+, human CD4, human CD8+, and human CD19 cells.

Results

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

Figure 2: Drug efficacy comparison in H358 tumor in SDR™ rats.

Figure 3: VCaP tumor kinetics in the SDR™ rat. VCaP cancer cells known to have poor engraftment rates and kinetics in immunodeficient mice, thrived in the SDR™ rat. The cells were inoculated in the flank region at a density of 106 cells per rat in a 1:1 solution of cell culture media and 5mg/ml Cxtrux. Engraftment rate was 75%. Each line represents growth in a single animal.

Figure 4: Drug efficacy in VCaP in SDR™ rats

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

Conclusions

1. Both the SDR™ and the SDR® rat models support the growth of various human cancer cell lines.
2. The NSCLC KRAS mutant cell line H358 has 100% take rate 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, had a very high engraftment and growth kinetics in the SDR™ rat.
4. The SDR™ is permissive to immune humanization with PBMCs, which could provide a model for immunoncology efficacy studies.

Acknowledgements

We would like to thank Dr. Goutham Narla for the cell lines. Supported by NIGMS grant R03GM099206 and KSTC grant 184-512-15-219