A Rag2/Il2rg double-knockout rat supports engraftment of human immune system for immunotherapy-based cancer efficacy studies

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Abstract

Immune humanized mice have been valuable in the development of novel cancer immunotherapies and have demonstrated stronger efficacy when combined with standard of care chemotherapy. An immune humanized rat could provide several advantages over the currently available humanized mouse models, including supporting the growth of larger tumors for serial fine needle biopsies to assess immune infiltration and serial blood draws for assessing human immune development and tumor biomarkers in real-time throughout an efficacy study. Using the SRG (**S**prague Dawley **R**ag2 -/- Il2r**g** -/-) immunodeficient rat, we developed a novel autologous human skin and immune cells-humanized rat model by co-engrafting full-thickness human-fetal skin and autologous fetal lymphoid organoids under the kidney capsule along with intravenous injection of autologous fetal-liver derived hematopoietic stem cells, thus termed, human skin-immune system humanized rat model (hSIS-humanized rat). hSIS-humanized SRG rats support development of adult-like, full-thickness human skin and human lymphoid organoids along with human immune cells. Methicillin-resistant Staphylococcus aureus inoculation in the human skin results in infection and skin pathology, thus recapitulating clinical outcomes. This model will enable *in vivo* mechanistic studies for development and evaluation of novel therapeutics for skin infectious disease and may also provide a model for establishing skin grafts of patient-derived melanoma tumors to investigate melanoma metastasis and response to therapies. In addition, engrafting the rat with human lymphoid organs and human immune cells may provide a similar platform to the BLT mouse for immunotherapy studies. Finally, we have demonstrated humanization of the rat immune system using human PBMCs. Human CD45+, CD3+, and CD20+ cells can be found in the peripheral blood, spleen, and bone marrow of engrafted rats. These immune humanized rat models may be beneficial for evaluating immunotherapies in human cancer models, including assessment of immune cell infiltration through fine needle biopsies.

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. 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, 28, 56 and 70 days to assess circulating human CD45+, human CD3+, human CD4+, human CD8+ and human CD20+ cells.

Results

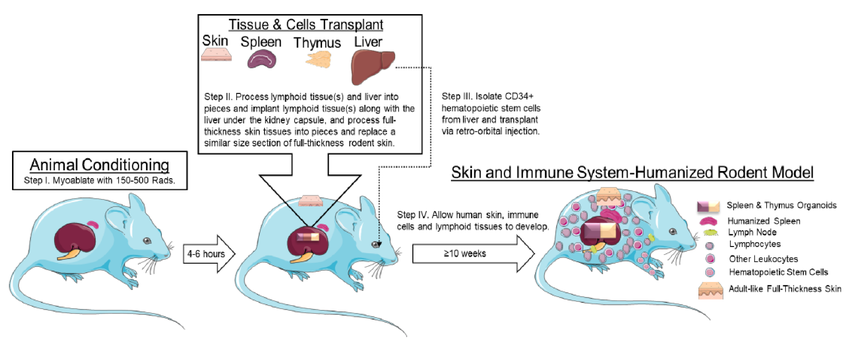

Figure 1. Construction of human Skin and Immune System-humanized rats. (I) Severely immunodeficient SRG rats were myoablated via gamma irradiation (II) Human fetal lymphoid tissue(s) and liver were processed into 1 mm^2 pieces and transplanted as a "sandwich" under the kidney capsule and full-thickness human fetal skin is transplanted on the panniculus carnosus of the dorsum in irradiated rodents, following the administration of antibiotics therapy and the induction of general anesthesia. (III) Autologous CD34+ hematopoietic stem cells were isolated from the fetal liver via magnetic selection and transplanted via retro-orbital injection following kidney capsule transplantation of the lymphoid tissues. (IV) Transplanted rodents were maintained under specific pathogen-free conditions and the human spleen and thymus organoids along with other lymphoid tissue(s) and associated immune cells are allowed to develop over a period of 10 weeks, resulting in the human Skin and Immune System-humanized rat model.

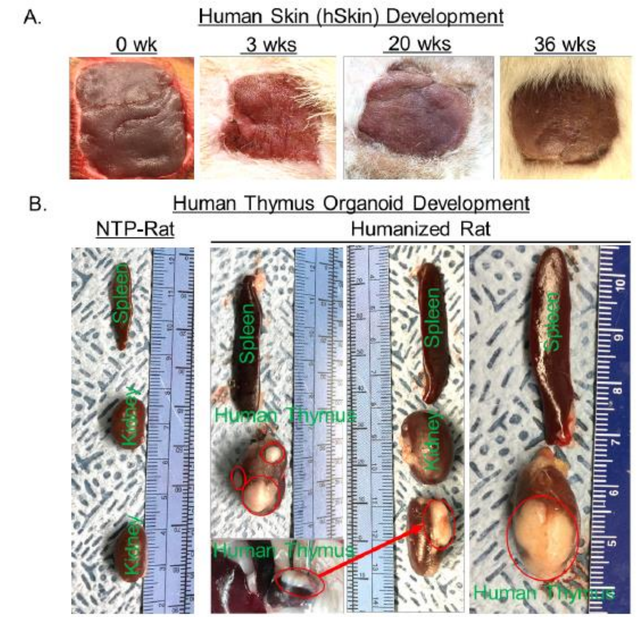

Figure 2. Development of human skin and lymphoid tissues in the human Skin and Immune System-humanized SRG rat model. (A) Representative gross-photos at 0 (the day of transplantation), 3, 20, and 36 weeks post-transplantation demonstrate robust human skin development. (B) Representative gross-photos of lymphoid tissues (human thymus in the kidney capsule and rat spleen) at 9 months post-transplantation demonstrates robust development of lymphoid tissues compared to non-transplanted SRG rats.

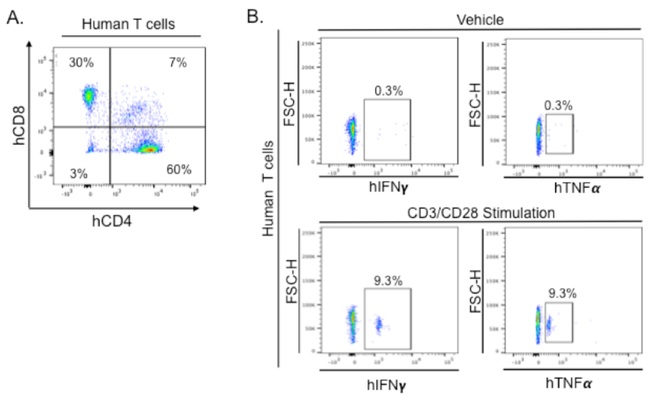

Figure 3. Human Skin and Immune System (hSIS)-humanized SRG rat model supports the development of functional human T cells. (A) Representative flow cytometry analysis of human T cells (hCD3+ cells) from the human thymus tissue of hSIS-humanized SRG rat at 36 weeks post-transplantation. (B). Flow cytometry analysis of cytokine response in human T cells from human thymus tissue following stimulation without (Vehicle) or with CD3/CD28 beads.

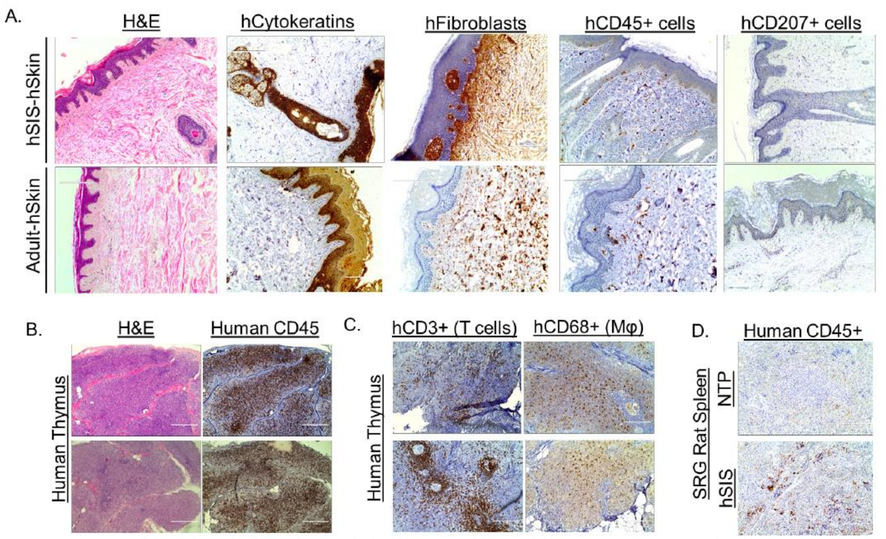

Figure 4: Immunohistochemical analysis of the human skin and immune system in the humanized SRG. (A). Various human skin cells are reconstituted in the human skin, including keratinocytes (AE1/AE3+cells, hCytokeratin+ cells), dermal fibroblast (TE7+ cells, hFibroblast+ cells), cutaneous immune cells (hCD45+ cells), and Langerhans cells (hCD207+). (B) Representative histological and immunohistochemical analysis of the human thymus (engrafted under the kidney capsule) demonstrate robust development of human thymus organoid at 9 months post transplantation, with human immune cells (Humans CD45+), including (C) high levels of T cells (hCD3+) and macrophages (hCD68+). (D) The rat spleen is also reconstituted with human immune cells (Humans CD45+); non-transplanted (NTP) SRG rat was used as a staining control. Scale bars: 200 µm.

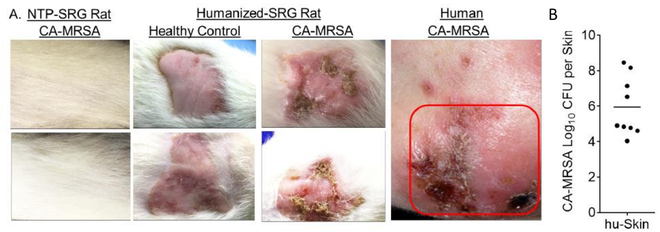

Figure 5. Human skin in SRG rats support CA-MRSA infection. CA-MRSA was inoculated (intradermal) into the engrafted human skin humanized SRG rats and the skin of non-transplanted (NTP) SRG rats (NTP-Rat). (A) Gross pathology was examined in CA-MRSA inoculated humanized rats and non- transplanted rats at 6 weeks post-inoculation, and compared to healthy control-human skin in humanized rats; (A) CA-MRSA infected human skin was used for comparative analysis of gross pathology in humanized rats and humans (Patient CA-MRSA skin photo credit: S. Camazine). (B) The human skin in humanized rats support high CA-MRSA bacteria load as measured at 6 weeks post-infection. Scale bars: 200 µm.

Conclusions

1. The SRG rat supports the engraftment of human skin and lymphoid tissues.
2. Human skin xenografted on the SRG is susceptible to infection with MRSA.
3. Human skin engrafted on the SRG is well established and comprised of various human skin and immune cells.
4. Human T cells that have developed in the human thymus tissue within the skin and immune humanized-SRG are functional and respond to stimulation.

Acknowledgements

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