Sprague-Dawley Rag2 null Il2rgamma null SRG rat (*OncoRat®*) has enhanced tumor microenvironment in human prostate cancer xenografts.

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Abstract

Human tumor xenografts are an essential tool for understanding tumor biology, growth kinetics, and therapeutic efficacy. While these studies are most commonly done in immunocompromised mice, we have created a Sprague Dawley *Rag2* null, *Il2rgamma* null ***SRG™*** rat that is an excellent host for human xenografts (***OncoRat®***). Lacking B, T, and NK cells, the SRG rat readily supports the growth of multiple human cancer cell lines, including lines that do not engraft well or grow consistently in existing mouse models. The tumor microenvironment (TME) is a critical factor for supporting xenograft tumors, and the microenvironment of a human tumor grown in the rat has yet to be fully characterized. In this study, representing a collaborative effort between research institutions we discovered that the tumor microenvironment in the SRG rat is more robust and supportive of human tumor growth than the same cells grown in the SCID/NCr mice.

To characterize the differences in rat and mouse TME, the human prostate cancer cell line VCaP was grown in SCID/NCr mice and SRG rats. Formalin fixed paraffin embedded sections were stained using immunohistochemistry (IHC) for both rat and mouse tumor microenvironment markers. Collagen marker CD29, endothelial cell marker CD31, hematopoietic lineage marker CD45, smooth muscle actin, and stromal markers CD54 and vimentin were analyzed in both animal hosts. When applicable, staining was quantified via counting positive cells per high powered field of view.

The host TME for VCaP xenograft tumors grown in SRG rats, was more robust when compared to the same model grown in SCID mice. Specifically, comparing the same markers in SRG rat and SCID/NCr mouse hosts revealed significant differences with the TME being more defined and robust in the SRG rat derived tumors. Results demonstrated a significantly increased number of stromal cells per high powered field in the SRG rat when compared to tumors of the same cell line grown in the SCID/NCr mouse. These data also demonstrate more human tumor epithelial cell interactions within the TME of the SRG rat than in SCID/NCr mouse. This increased stromal involvement more accurately recapitulates the human TME and may help explain the better take rates and faster growth rates of xenografts in SRG rats versus SCID/NCr mice. It is well known that recapitulating the tumor cell population heterogeneity is a study limitation when using animal models. Utilization of the SRG rat TME has great value in nonclinical research by more accurately translating the human disease context, while remaining in a readily available immunodeficient animal model (i.e., OncoRat).

The SRG™ Rat

Hera BioLabs has generated an immunodeficient Sprague Dawley *Rag2* null, *Il2rgamma* null ***SRG™*** rat. SRG rats lack mature B, T, and circulating NK cells, rendering it the most immunodeficient rat available. With a unique need for larger animals that can support human tumor growth, the SRG continues to distinguish itself from immunocompromised mice.

While there are many immunodeficient animal models to host human cancer xenograft tumors, there are a number of cancer cell lines like VCaP that grow irregularly with poor take rates and tumor growth kinetics. However, in the SRG rat, VCaP tumors have between 90-100% engraftment rates, with uniform growth kinetics and large tumor volumes can be supported (1).

The larger size of the rat enables increased tumor volumes, in addition to improved pharmacokinetic, pharmacodynamic, and toxicology parameters that come with the larger size and more robust nature of the rat. The SRG rat is extremely permissive to hosting human cancer cell lines and is a first in the field of immunocompromised rats for use in preclinical oncology.

Materials and Methods

Establishment of human prostate cancer xenograft tumors: VCaP tumors were inoculated into either SCID/NCr mice or SRG rats (5 million and 10 million cells, respectively). Cells were mixed with Matrigel® 1:1 and injected 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 animal host proteins: Tumors were excised and fixed in 10% NBF. Standard 5um sections were collected and mouse/rat markers were visualized via antibody staining (CD29: rabbit anti-mouse, human, rat; AbCam Ab183666. CD45: Rabbit anti human, rat, mouse; AbCam Ab208022. SMA: rabbit anti mouse, human, rat; AbCam Ab32575. Vimentin: Rabbit anti mouse, human, rat; AbCam Ab92547). Human tumor cells were visualized with hematoxylin counterstain. Positive cells per mm^2 high powered field of view were counted in addition to percentage of visual field for quantification, and statistical significance was determined by unpaired t-test.

Results

Figure 1: Characterization of the host tumor microenvironment into VCaP xenografts in SCID mice and SRG rats.

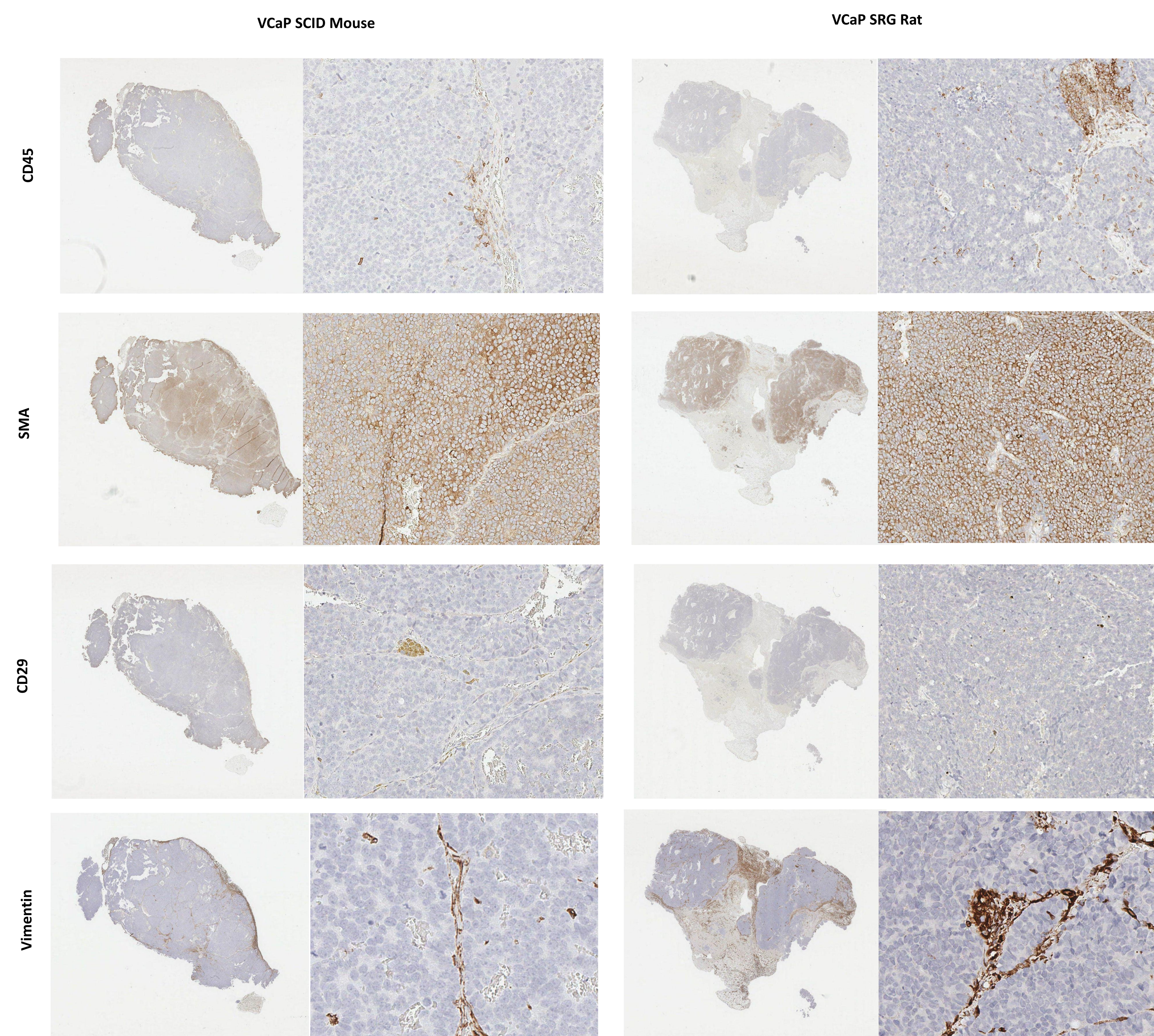

Figure 1. Representative images of the TME in mouse and rat xenografts injected subcutaneously with VCaP cells. SCID/NCr mice and SRG rats were inoculated with 5x10^6 and 10x10^6 VCaP cells, respectively, subcutaneously in the hind flank. Immunohistochemical (IHC) staining of tumor tissue for CD45, SMA, CD29, and Vimentin.

Figure 2: Key differences in microenvironment markers in SRG rats.

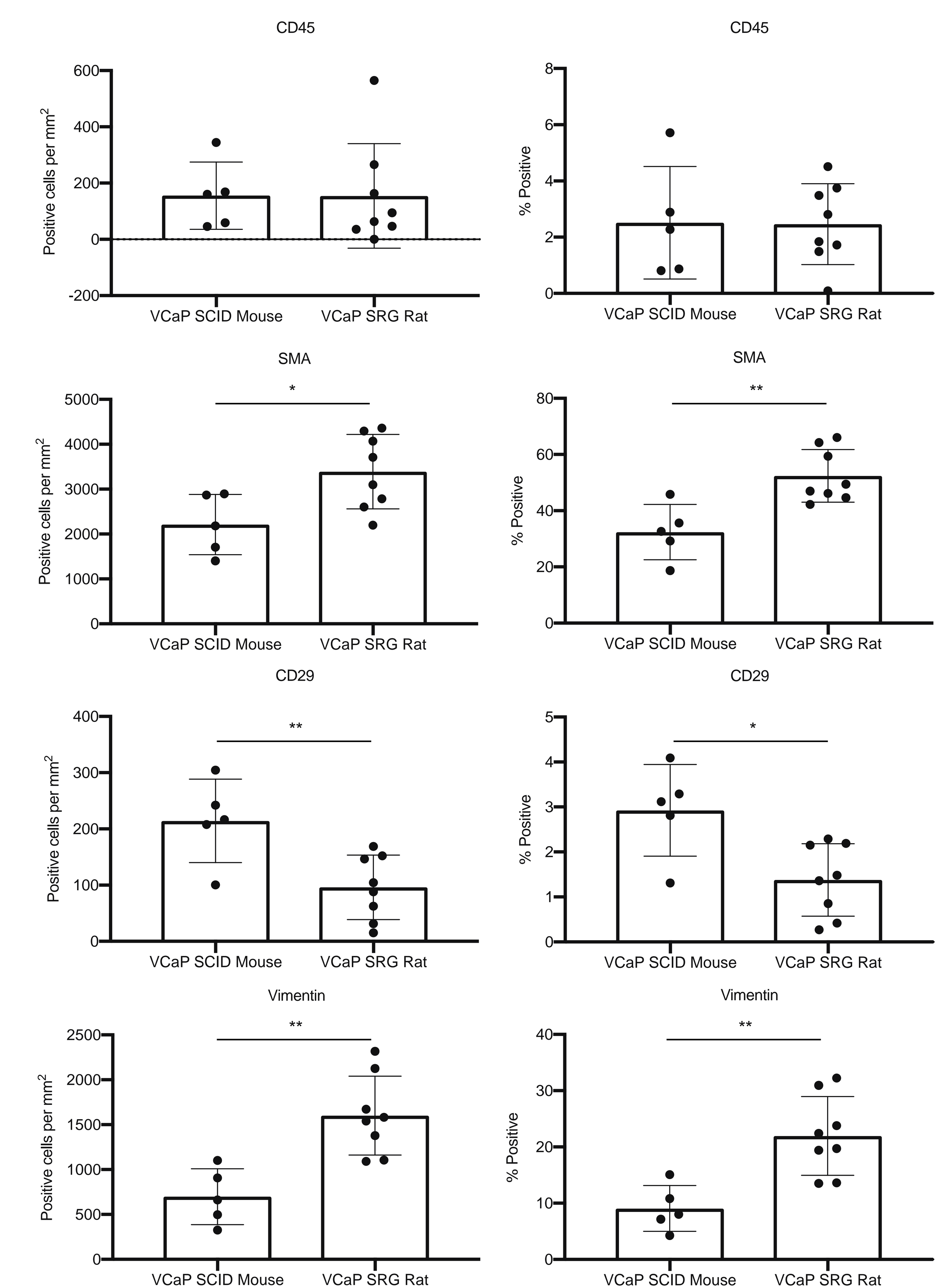

Figure 2. Quantification of IHC representing positive cells per mm^2 and percent positive cells. (Unpaired t-test, p-values: * < 0.05, ** <0.01).

Conclusions & Future Research

Conclusions

1. The Stromal markers vimentin and smooth muscle actin are significantly increased in SRG rat microenvironment when compared to SCID mouse. Increased heterogeneity from stromal involvement within the VCaP xenograft tumor more closely recapitulates the tumor microenvironment found in clinical settings.
2. There is low overall incidence of both CD29 and CD45 positivity, indicating low integrin complexes and collagen presence (CD29) as well as low hematopoietic lineage cells (CD45).
3. The increased stromal invasion may contribute to the SRG's unique capability to support xenograft tumors that are classically challenging to engraft.

Future Research

Hera BioLabs, in collaboration with the University of Michigan, will continue to characterize the tumor microenvironment in SRG rats when compared to immunocompromised mice. We are focused on quantifying the tumor vasculature within the xenograft tumor via CD31 staining and further stromal markers via CD54 staining.

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References

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