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Blockade of B2 –GPI decreases hypoxic tissue damage

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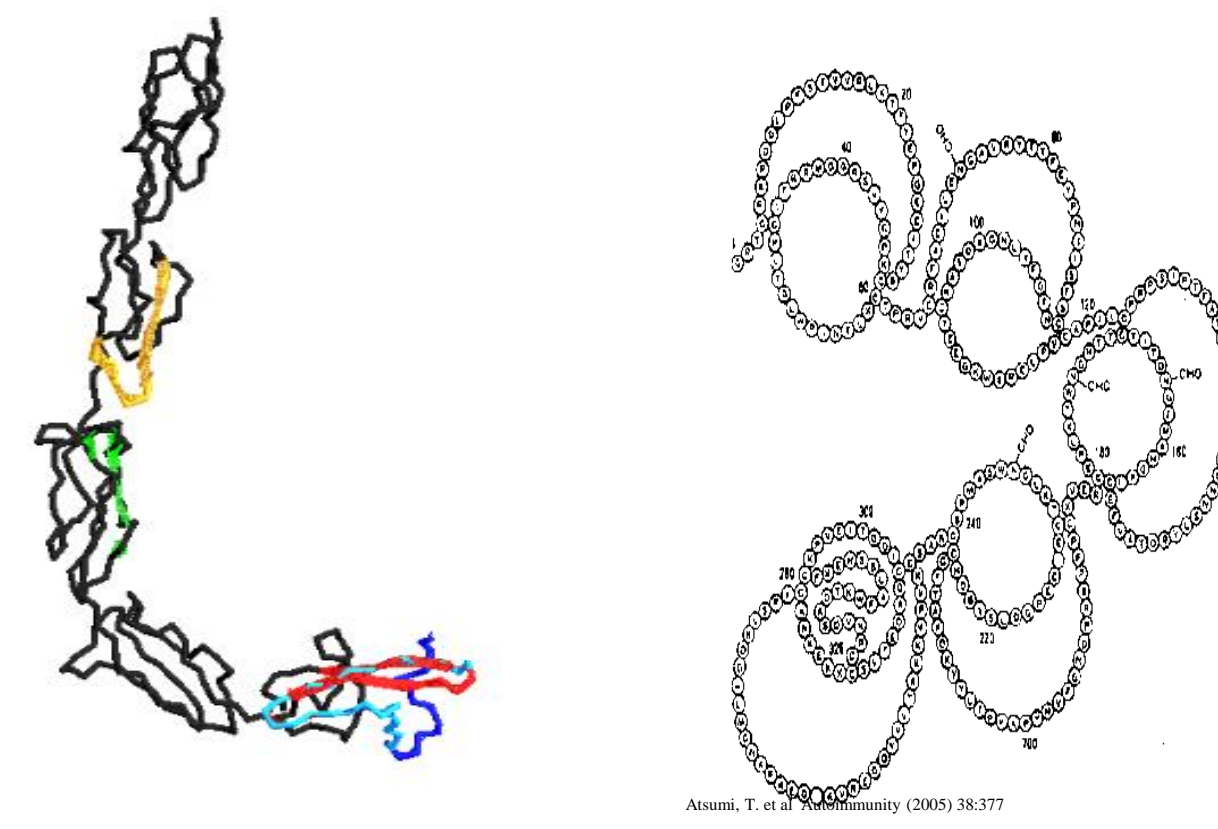
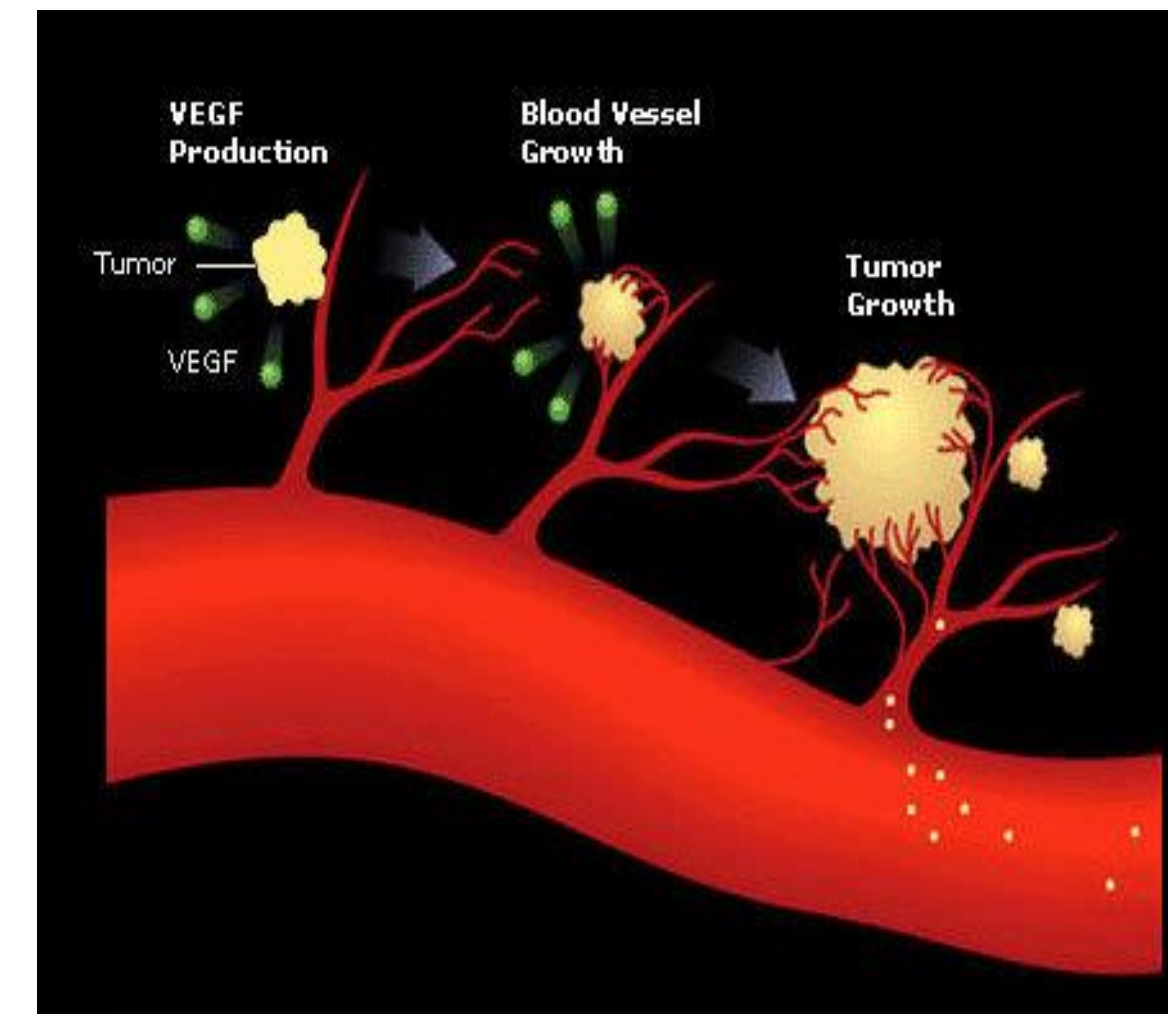
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Introduction

The risk of melanoma, a form of skin cancer, increases with age but tanning beds and other forms of tanning increase the risk for young people too. This deadly cancer kills almost 10,000 people each year. Skin cancer tumors develop in melanocytes, which produce the skin pigment. Tumor growth primarily depends on the growth of new blood vessels (angiogenesis).

β 2-GPI, a protein in high concentration in the blood, binds to hypoxic tissues or damaged endothelial cells. Studies have shown β 2-GPI can induce or inhibit angiogenesis. Peptides 296c-s and RD-p9 were derived from the binding region of β 2-GPI (red line) to inhibit β 2-GPI activity.

C3 complement activation is a part of complement cascade that begins inflammation and is central to complement activation when the antibody, IgM is present. IgM activates the classical complement pathway and is one of the isotypes in the initial antigen-antibody complexes. In male mice tissue damage is via IgM recognition of β 2-GPI with complement activation. Clinical studies and other animal models indicate sex hormones alter vessel dilation during reperfusion β 2-GPI binding and complement activation may differ by sex due to different anatomy between genders. We hypothesize that β 2-GPI, C3, and IgM all play a significant role in melanoma cancer. By blocking β 2-GPI we can significantly decrease melanoma tumor growth.

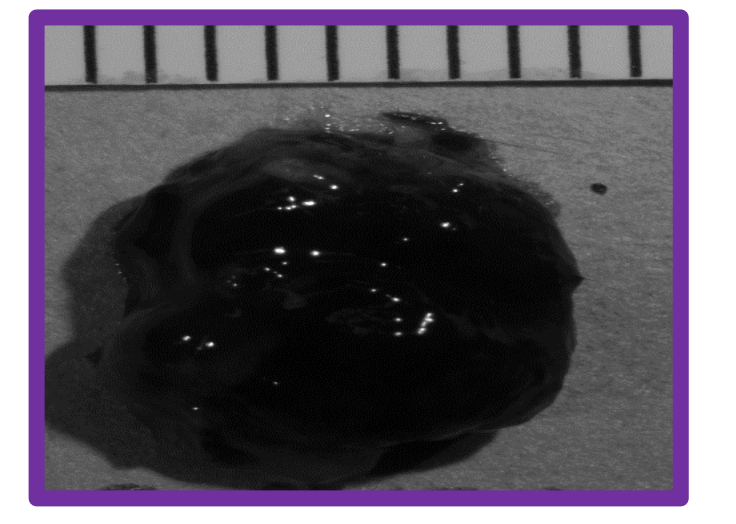


Melanoma Tumor Size Varies by Sex and β 2-GPI Peptides

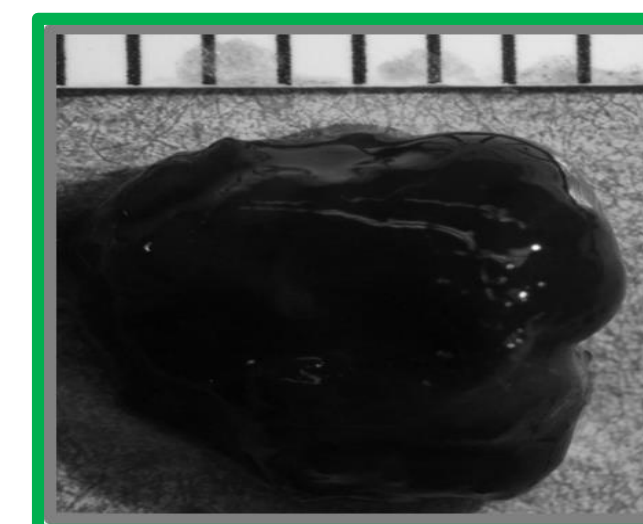
Male Saline



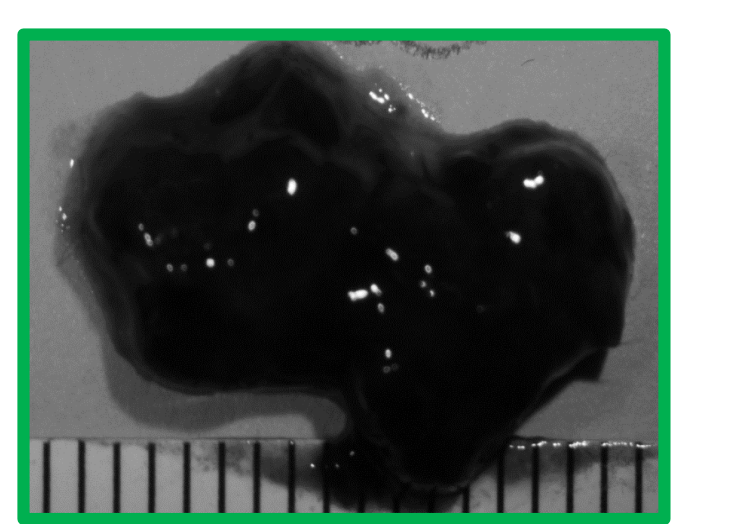
Female Saline



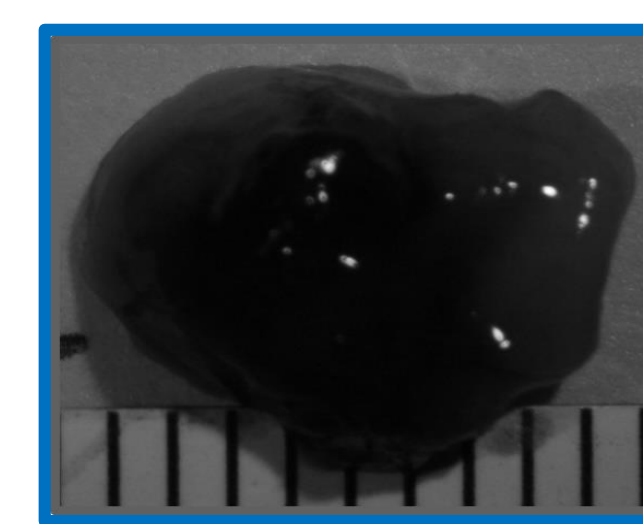
Male p16ss



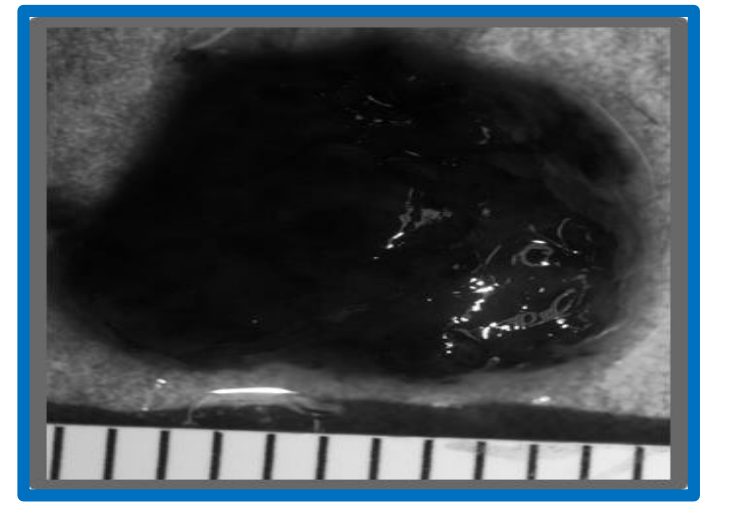
Female p16ss



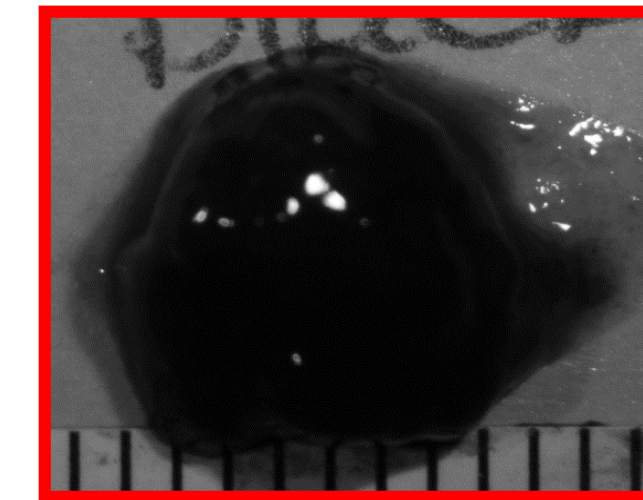
Male RDp9



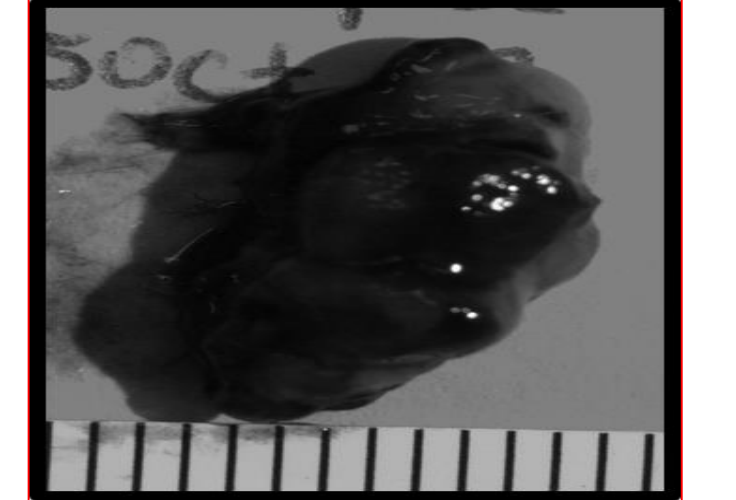
Female RDp-9



Male 296c-s



Female 296c-s



On the 10th day the melanoma tumors were removed from each mice. Tumor pictures were recorded for each tumor. Tumors treated with saline and p16ss appear to be significantly larger than the tumors treated with the blocking peptides 296c-s and RD-p9. Representative of 4-6 tumors per peptide.

Hypothesis

β 2-GPI plays a role in hypoxic tissue damage and that blocking β 2-GPI may decrease tumor growth.

Methods

Cells:

B16F10 were cultured in DMEM2 with 5% Nu -Serum, 5% FBS, 10% vOpti-mem, and 1x Glutamax in a humidifier incubator (37°C) with 5% CO₂. They were split 1:5 Mondays and Wednesdays and 1:10 on Fridays.

Melanoma Tumors:

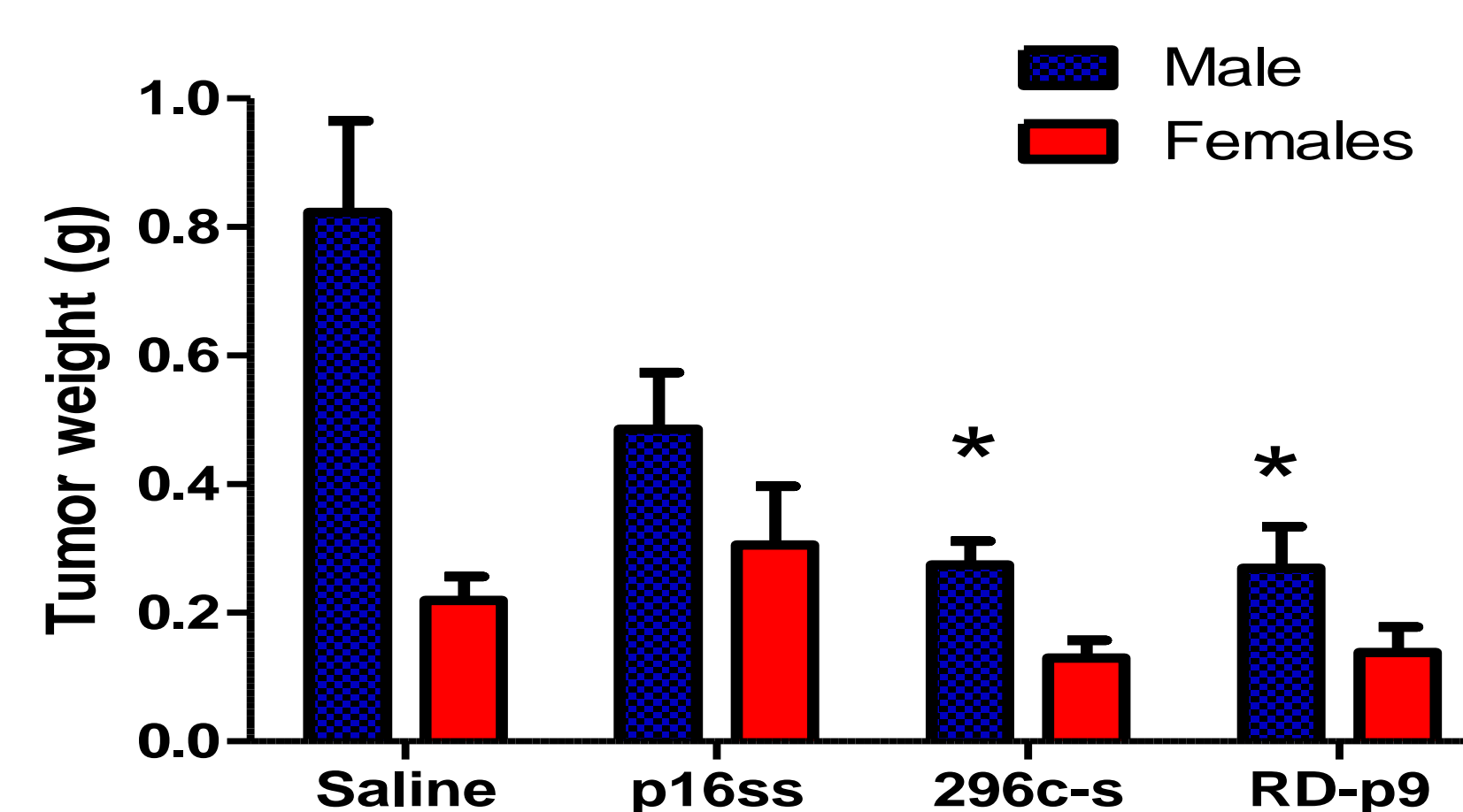
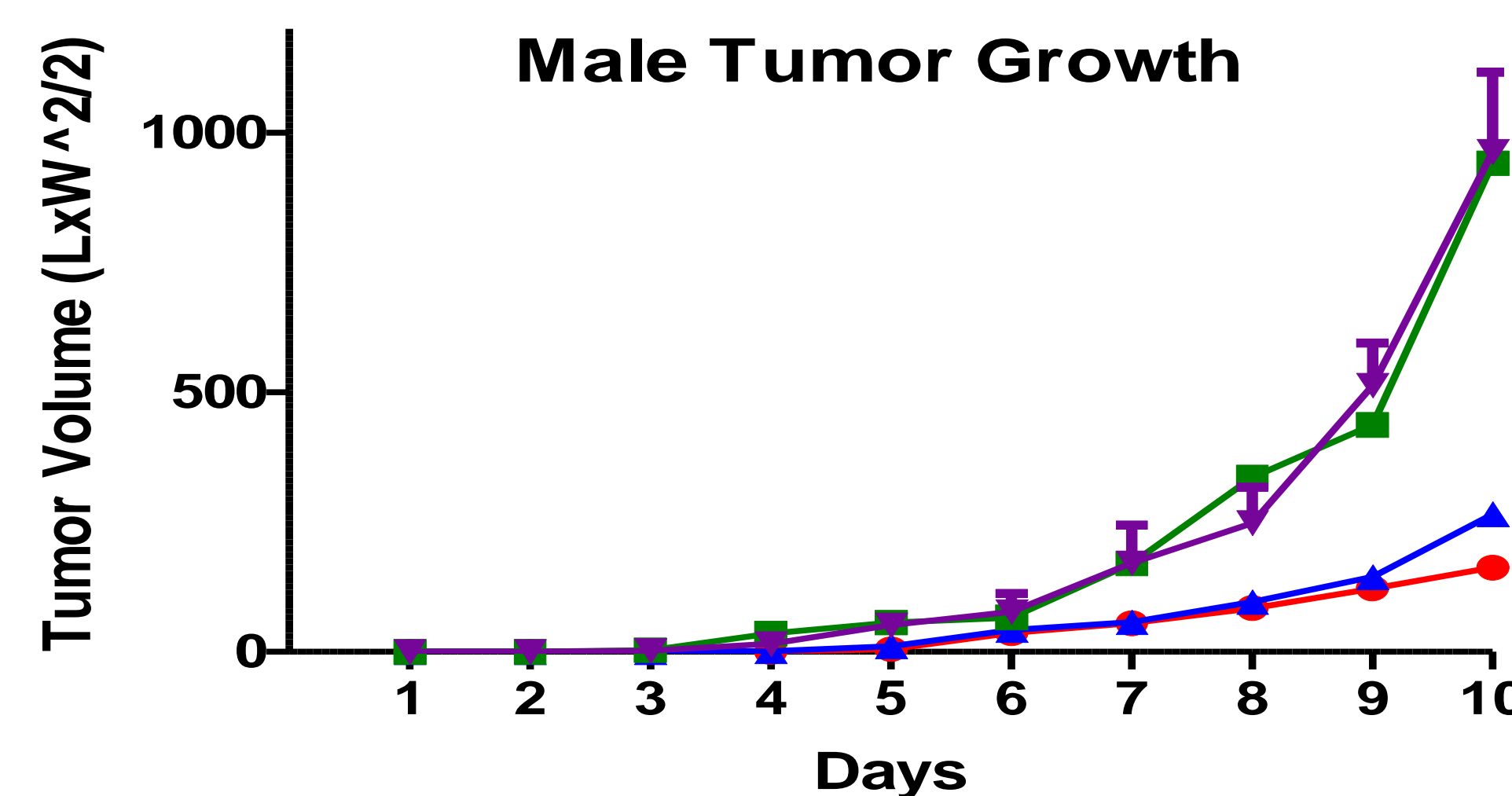
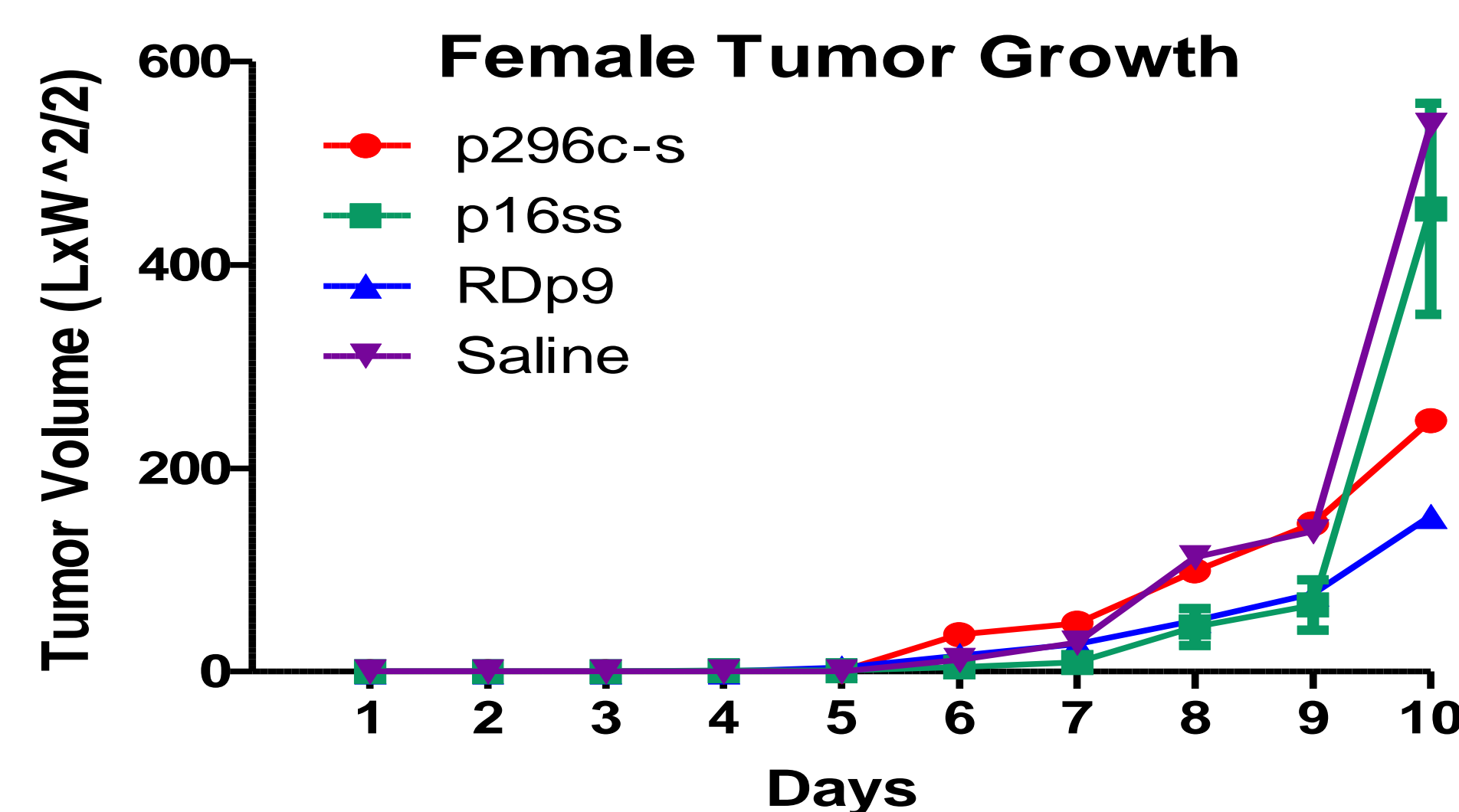
C57Bl/6 mice (6-9 weeks old) were bred and maintained in the Division of Biology at K-State. Mice were kept in a 12 hour light-dark cycle with constant access to rodent food and water. The Institutional Animal Care and Use Committee (IACUC) approved all procedures and complied with the Animal Welfare Act. Mice were injected with 2×10^6 B16F10 cells in Matrigel (1:1) and tumors were measured daily for 10 days prior to removing tumors. Peptides were given to mice by retro-orbital injection on multiple days during the 10 day period. Tumor weight and pictures were recorded of each animal after removal.

β 2-GPI and C3 and IgM Immunohistochemistry:

Tissues were incubated with 10% Donkey sera in PBS for 30 minutes at 37°C to block non-specific antibody binding. Tissues were covered with a primary antibody or an appropriate isotype control antibody. The primary antibody is specific for the antigen and the isotype antibody serves as a negative control and is not specific. After washing, a secondary antibody is added to the slides at room temperature and in darkness to prevent fluorescence wash out. Slides were then cover slipped. Pictures were of the taken of the melanoma tumors using Infinity Analyze program and a Nikon Eclipse 80i fluorescent microscope.

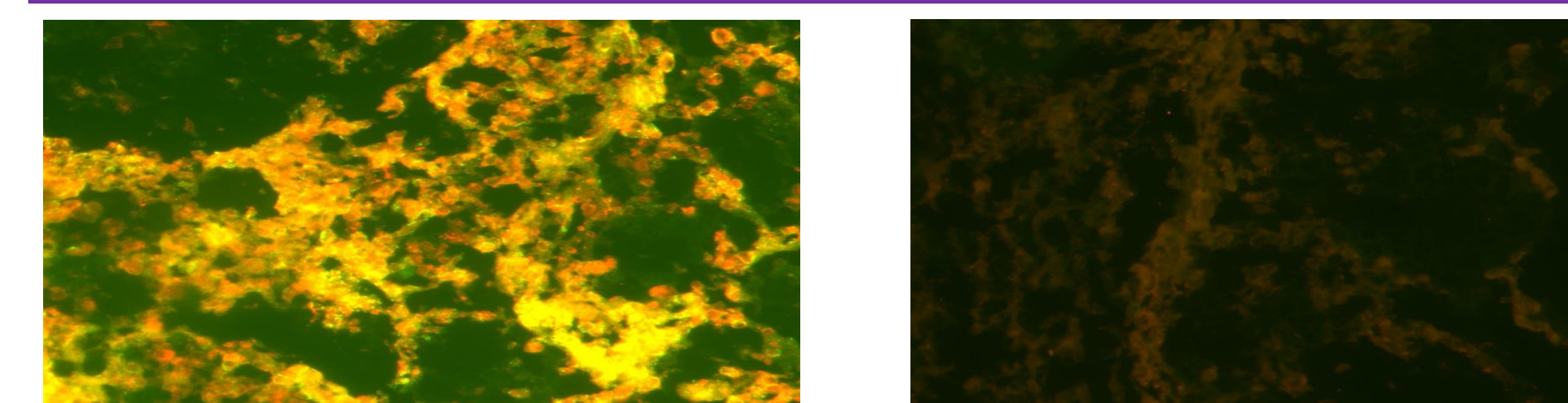
Results

β 2-GPI Peptides Decreased Melanoma Tumor Size



Mice were injected with 2×10^6 B16F10 cells and tumors were measured each day for 10 days until removed. Tumors were measured daily over the course of 10 days. The weight of each tumor was recorded on the 10th day after the removal of the tumor from the mouse. Peptides 296c-s and RD-p9 showed a significant decrease in tumor growth compared to the controls saline and p16ss. Males demonstrated a larger growth in both volume and weight. Representative of 4-7 tumors per peptide.

Female Melanoma Tumors Express C3 and IgM



Melanoma Tumors express C3 and IgM by Immunohistochemistry. C3 and IgM demonstrated bright fluorescent co-localization on the tumors that were stained with both the primary and secondary antibodies. The tumors stained with control antibodies displayed a very dim fluorescent. This suggest C3 and IgM are both present within a melanoma tumor. Representative of 4 tumors with 6-8 pictures per tumor.

Conclusion

- Male tumors grew faster and larger than female tumors.
- Peptides 296c-s and RD-p9 decreased melanoma tumor growth and weight suggesting therapeutic potential.
- Both C3 and IgM are present in female melanoma tumors.

Future Directions

- Increase sample size for C3 and IgM immunohistochemistry.
- Compare C3 and IgM in tumors from males vs females.
- Conduct more C3 and IgM IHC with tumors treated with and without peptides.

Acknowledgements

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