Localization and Expression Level of Vascular Endothelial Growth Factor after Partial Hepatectomy of WKY Rats

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Background

- Inflammation and fibrosis are indicative of liver regeneration following injury and chronic liver diseases, such as cirrhosis and hepatocellular carcinoma
- The formation of new vasculature via the process of angiogenesis is vital to the pathological progression of liver regeneration and these diseases
- Vascular endothelial growth factor (VEGF) is the most potent and specific growth factor for initiating the process of angiogenesis
- Bevacizumab is an antibody that binds to VEGF, inhibiting it from initiating angiogenesis.

Previous Study

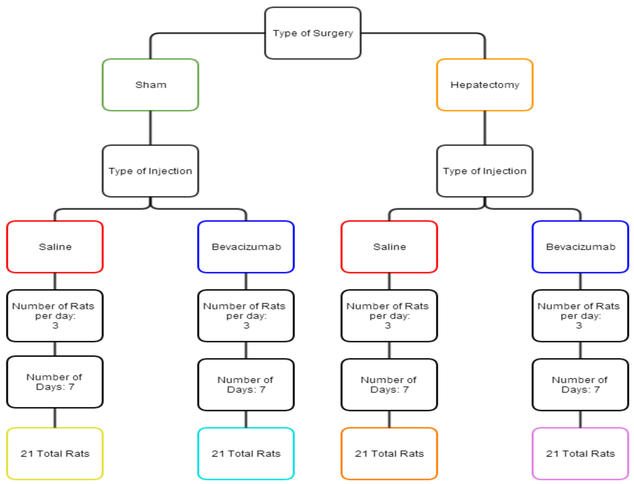

Figure 1: Flow chart of surgeries and injections

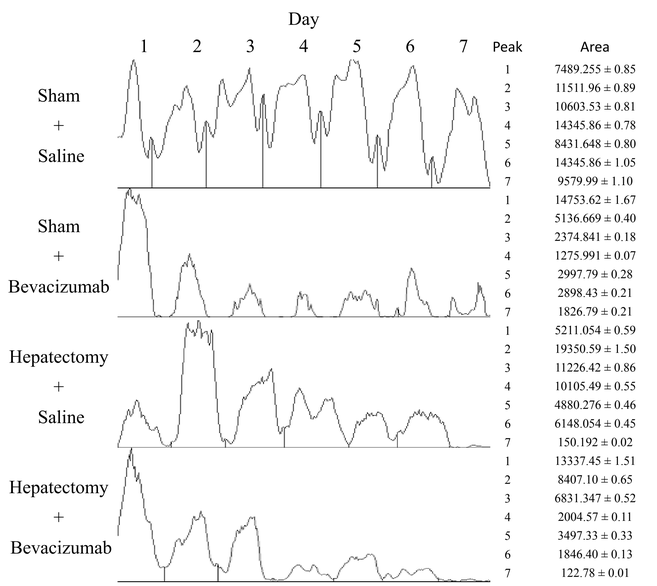

Figure 2: Image J densitometry analysis of Western Blots for all treatments, normalized to the nitrocellulose protein expression. The areas under the peak are displayed right of the figures and are measured in pixels.

Results

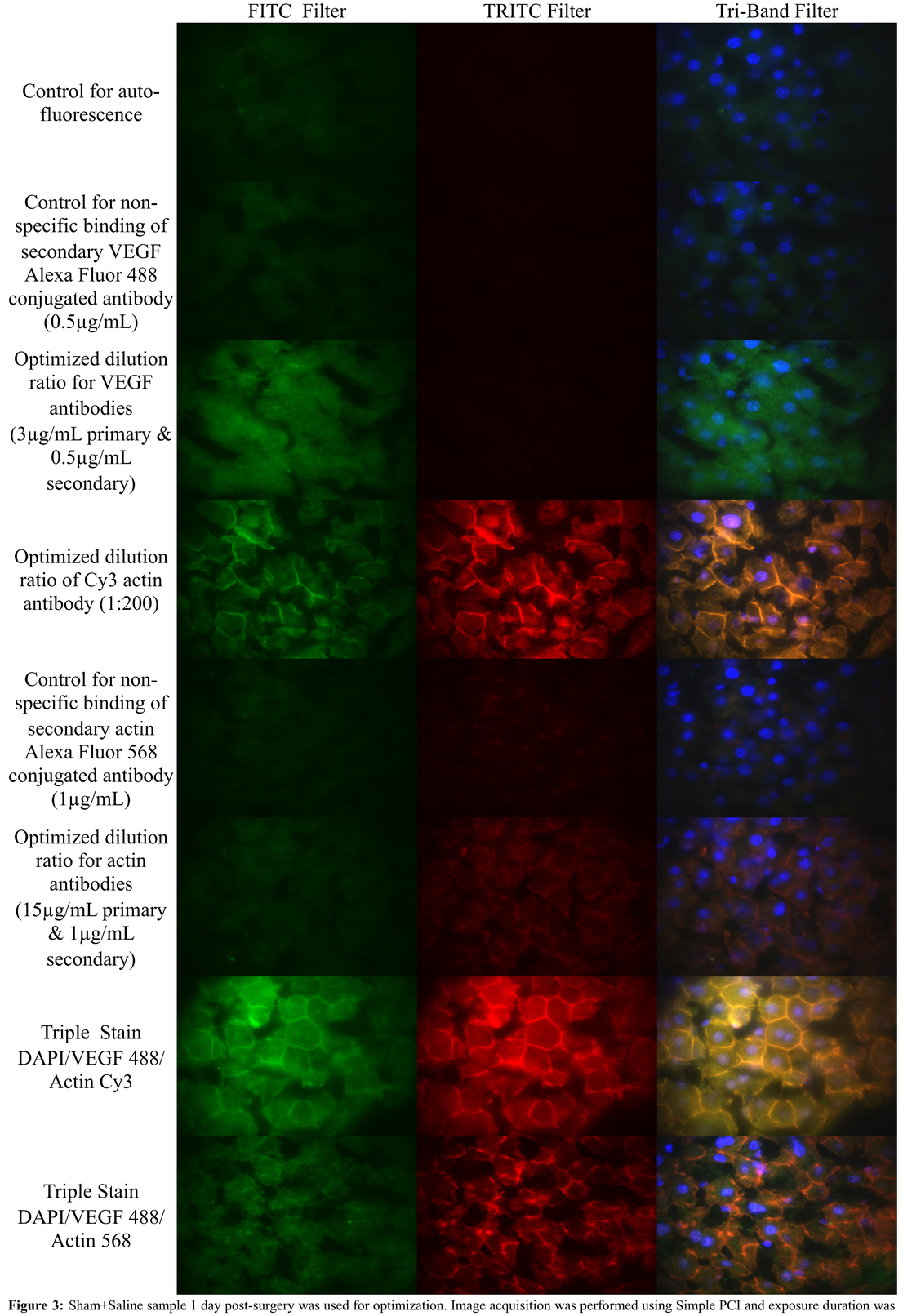

Figure 3: Sham+Saline sample 1 day post-surgery was used for optimization. Image acquisition was performed using Simple PCI and exposure duration was kept consistent for each fluorophore as follows: DAPI at 0.4 sec, Alexa Fluor 488 at 6.0 sec, Alexa Fluor 568 at 1.0 sec, and Cy3 at 0.55 sec.

Current Study

Phase 1: Titration of antibodies to determine the dilution ratio of each antibody

- VEGF antibodies:

  Primary—Rabbit Anti-Rat VEGF / Secondary—Goat Anti-Rabbit conjugated with Alexa Fluor 488
- Actin antibodies

  Option 1: Monoclonal anti-actin antibody produced in mouse conjugated with Cy3

  Option 2: Primary—Monoclonal Anti-Actin produced in mouse / Secondary—Goat Anti-Mouse conjugated with Alexa Fluor 568

Phase 2: Acquire digital images for each of the 4 groups over the 7 days and evaluate them using Image J to determine the localization and expression level of VEGF.

Methods

1)Samples sectioned at a thickness of 7-8 microns and collected on Histobond slides

2)Rinsed in PBS for 5 mins at room temp

3)Fixed with 0.1% paraformaldehyde for 15 min at room temp

4) Quenched excess paraformaldehyde with ethanolamine for 10 min at room temp

5)Tissue was permeabilized in chilled methanol and incubated for 5 min at -20˚C

6)Washed 5 mins then blocked with 1% BSA, 1% inactivated FCS, and 0.3M glycine in PBS for 30 min at room temp

7)Incubated with primary antibodies diluted in 1% BSA, 1% inactivated FCS block overnight at 4˚C

8)Washed 3 times in PBS for 15min/wash

9)Incubated with secondary antibody, diluted in 1% BSA, 1% inactivated FCS block, that was filtered with a 0.45 micron filter for 2-3 hours at room temp

10)Washed 3 times in PBS

11)Coverslips were mounted using Prolong Gold Antifade media with DAPI and sealed with clear nail polish

Future Studies

Quantitative Reverse Transcription Polymerase Chain Reaction assay will be used to determine gene expression of VEGF for each of the four groups over the 7 days.

References

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