Mar 29th, 4:00 PM - 5:00 PM

Exploring a Link Between Spy1 and Hepatocellular Carcinoma Progression

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Spy1 and Hepatocellular Carcinoma Progression:

Exploring a Link in a Murine Model

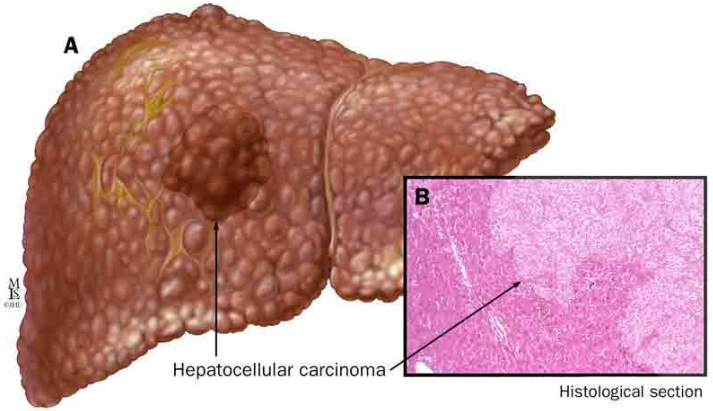
Presented by : Carlee Stoyanovich

Honors Thesis Project 2016

University of Windsor

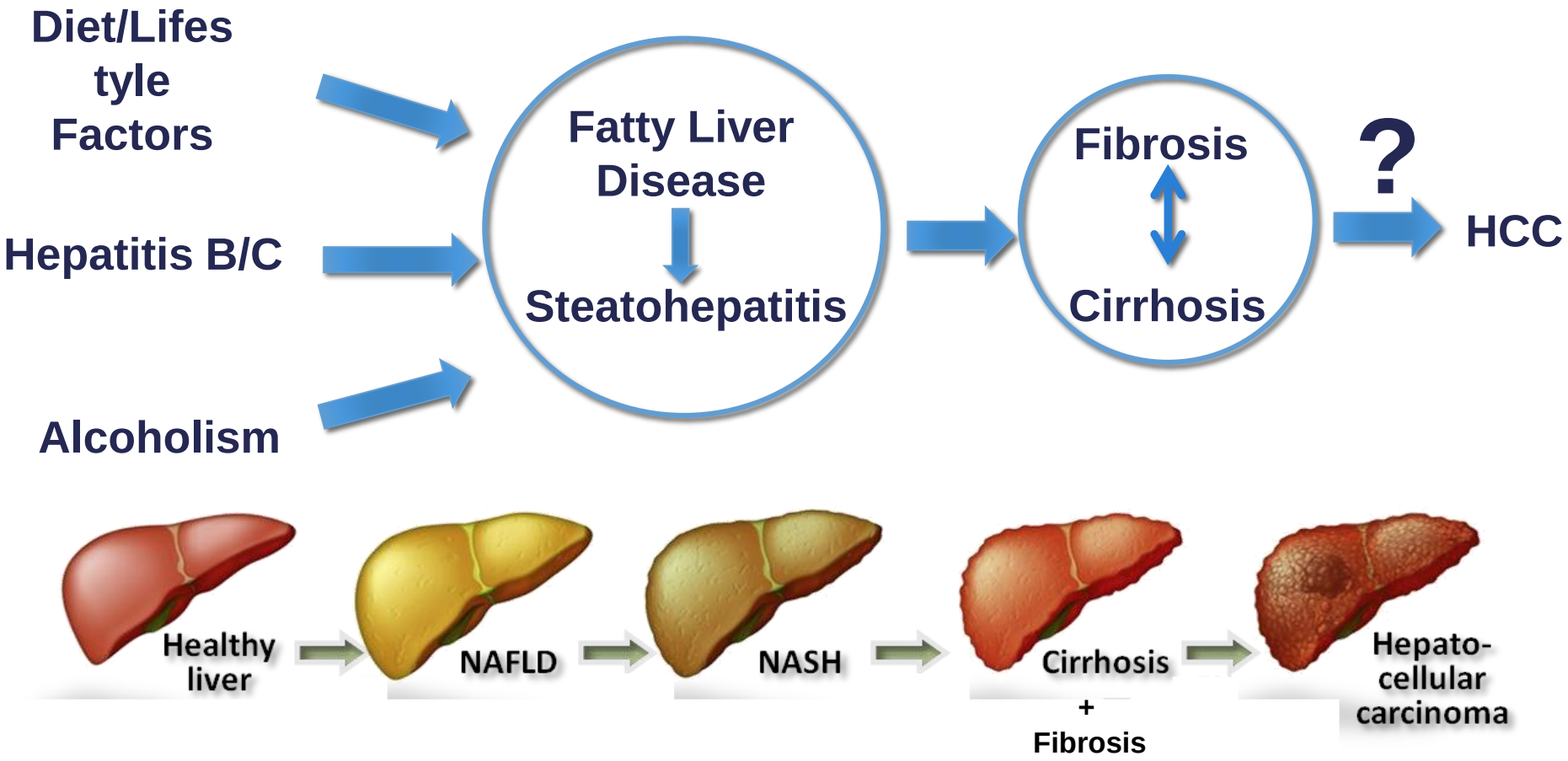
Hepatocellular Carcinoma (HCC)

- The most aggressive and prevalent form of primary liver cancer
- In men, it is the 5th most common cancer and in women it is the 7th worldwide
- Current treatments are invasive and include: transplantation, resection, ablation and chemotherapy
- The 5-year survival rate is 20%



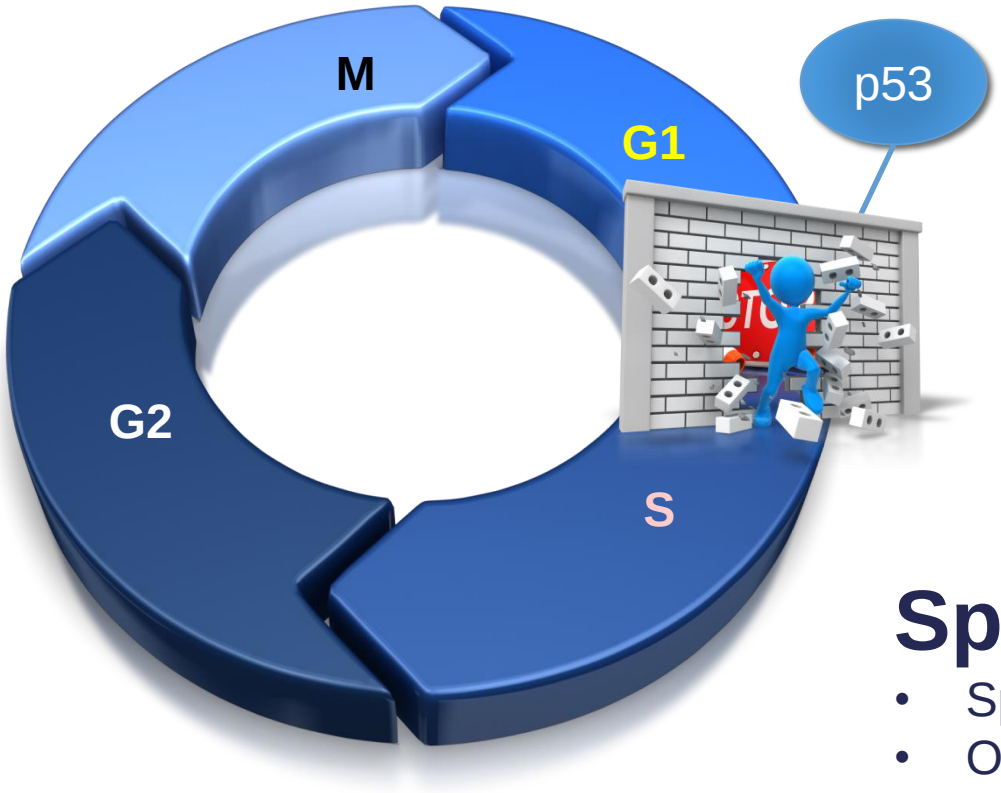
http://www.hopkinsmedicine.org/liver_tumor_center/conditions/cancerous_liver_tumors/hepatocellular_carcinoma.html

The Progression



http://www.bionapcfa.com/2015/11/can-fite-advances-cf102-in-massive-nash.html

A Protective Mechanism



p53

- Tumor suppressor
- Halts the cell cycle during unfavorable conditions
- Regulates cell death (apoptosis)
- Aids in DNA repair
- In HCC normally inactivated or mutated

Spy1

- Speed up cell division
- Override cell cycle barriers
- Enhance stemness in cell populations
- Known role in breast and brain cancers

MMTV-Spy1 Mouse

B6CBAF1/J genetic background

- Designed to study breast cancer in mice models
- Constitutively overexpress Spy1 in the mammary gland

HCC in the Spy1 Mouse Model

- MMTV-Spy1 male mice with high levels of Spy1 have significantly more HCC than their male littermate controls.

Contents lists available at ScienceDirect

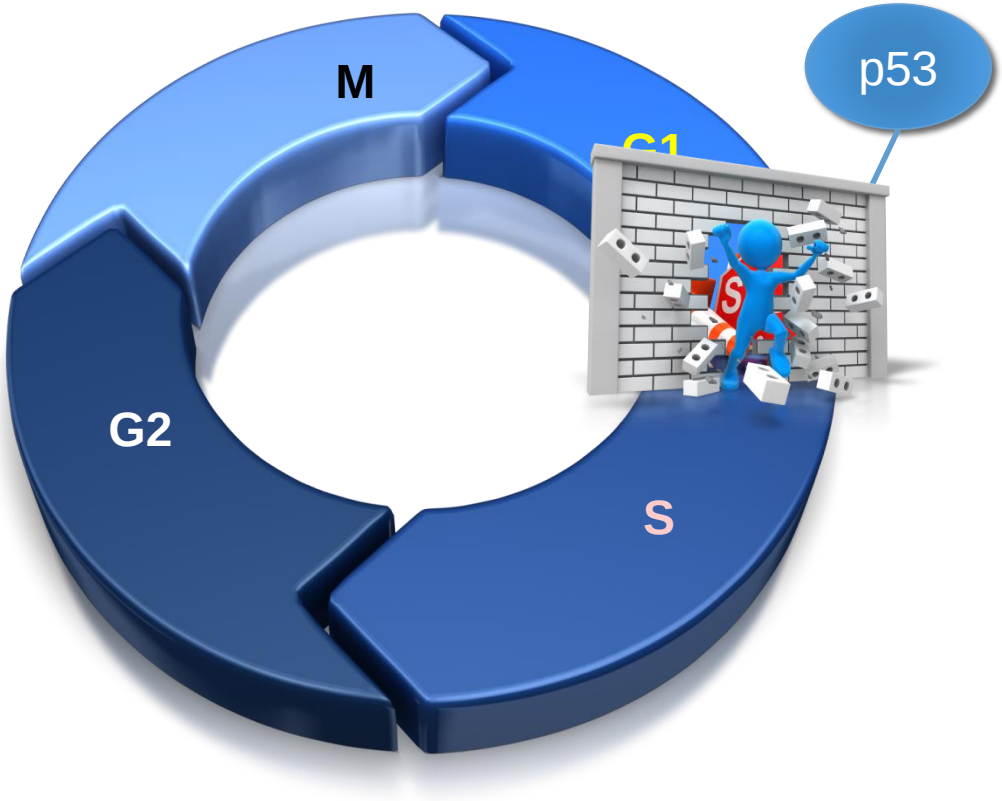
Experimental and Molecular Pathology

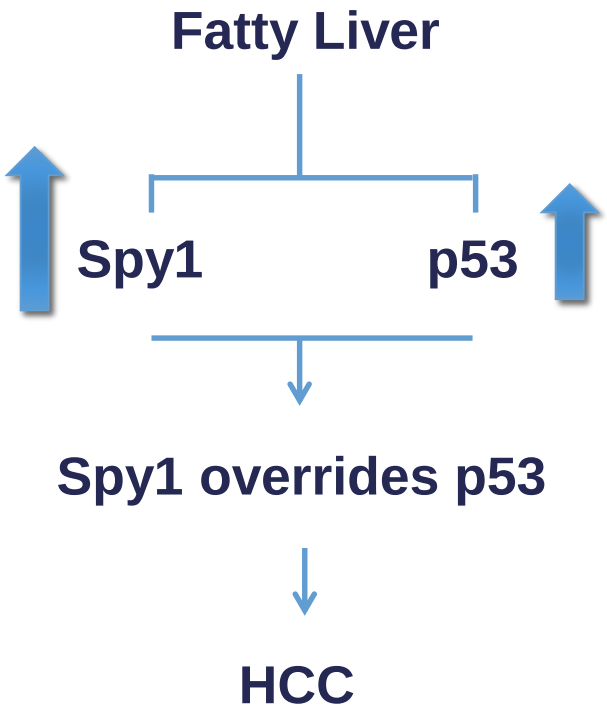
journal homepage: www.elsevier.com/locate/yexmp

Expression and prognostic role of Spy1 as a novel cell cycle protein in hepatocellular carcinoma

Qing Ke [a,1], Juling Ji [a,1], Chun Cheng [a], Yixin Zhang [a], Mudan Lu [d], You Wang [d], Li Zhang [a], Peng Li [c], Xiaopeng Cui [c], Li Chen [a], Song He [a,*], Aiguo Shen [b,*]

A Potential Mechanism?

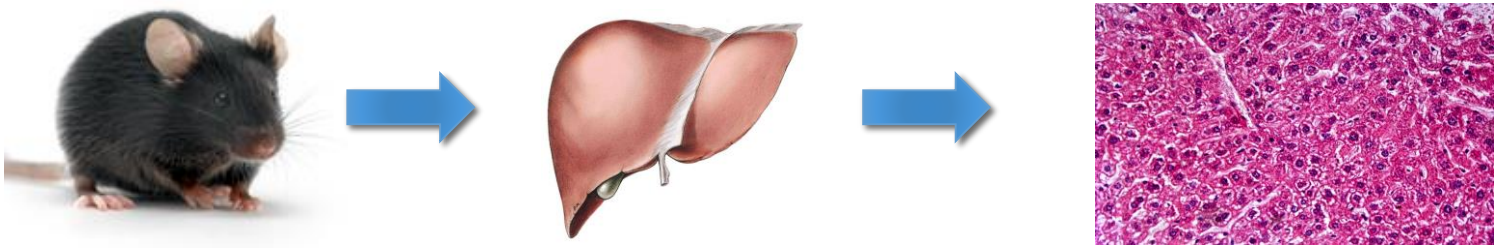




Does an increase in Spy1 levels predispose the liver to HCC development?

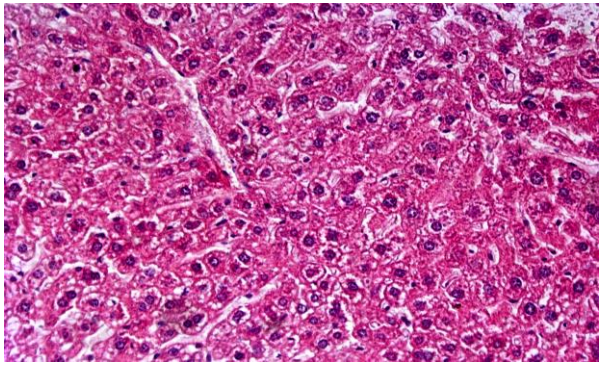
Objectives

- Further characterize the MMTV-Spy1 liver phenotype.
- Develop a model to look at HCC progression in wild-type mice.
- Quantify Spy1 protein levels in the wild-type damaged mice livers.
- Monitor fat accumulation as well as p53 and TNF-alpha levels in the mice livers.



Effects of Spy1 on Liver Morphology

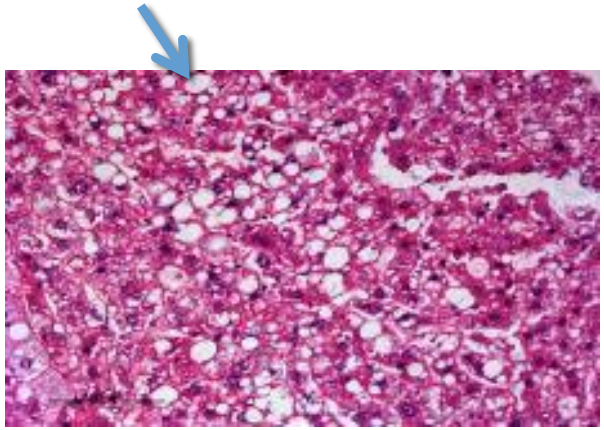
Normal hepatocytes



Healthy liver

+1yr control mouse

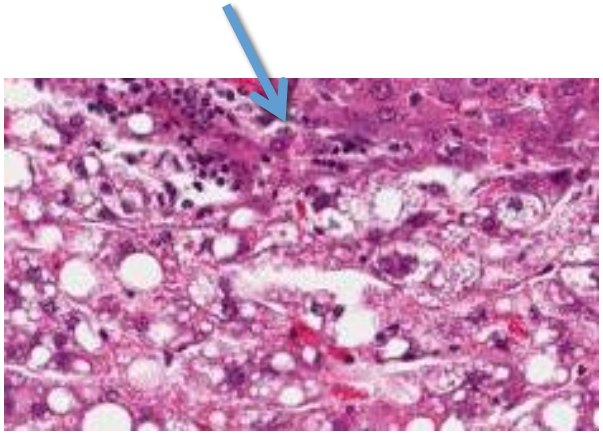
Large vacuoles



Fat accumulation

+1yr MMTV-Spy1 mouse

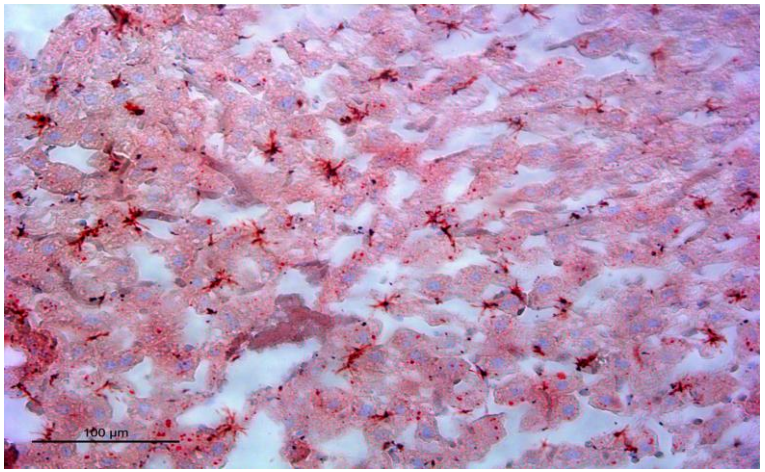
Disordered cell structure



HCC

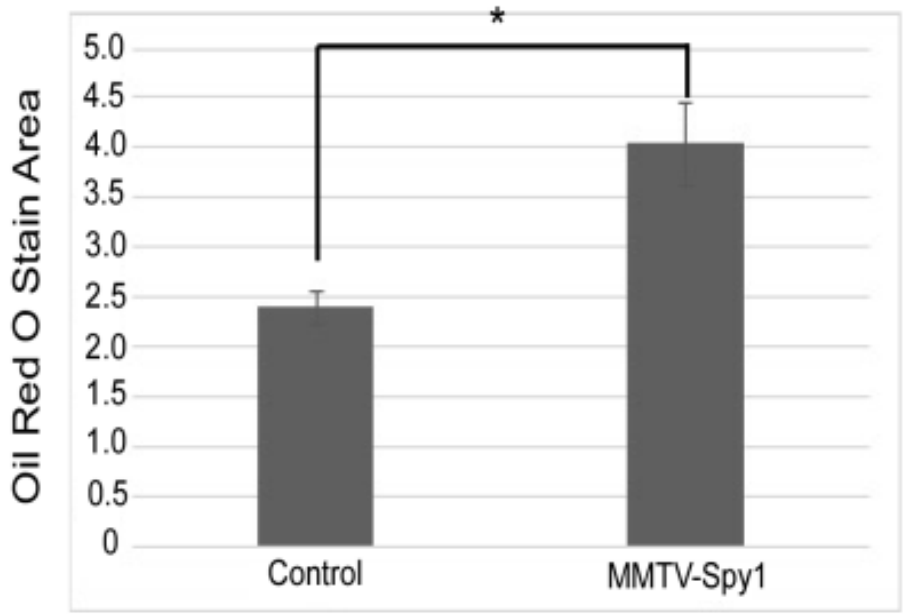
+1yr MMTV-Spy1 mouse

Fat Accumulation in MMTV-Spy1 Mice



10 month control mouse

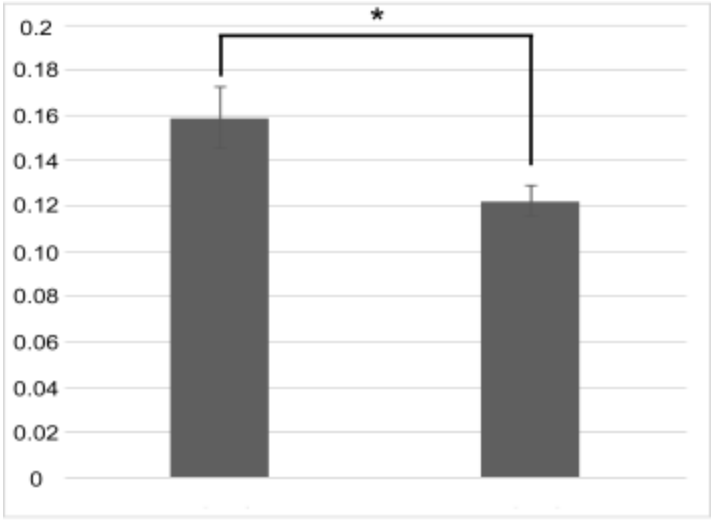
10 month MMTV-Spy1 mouse

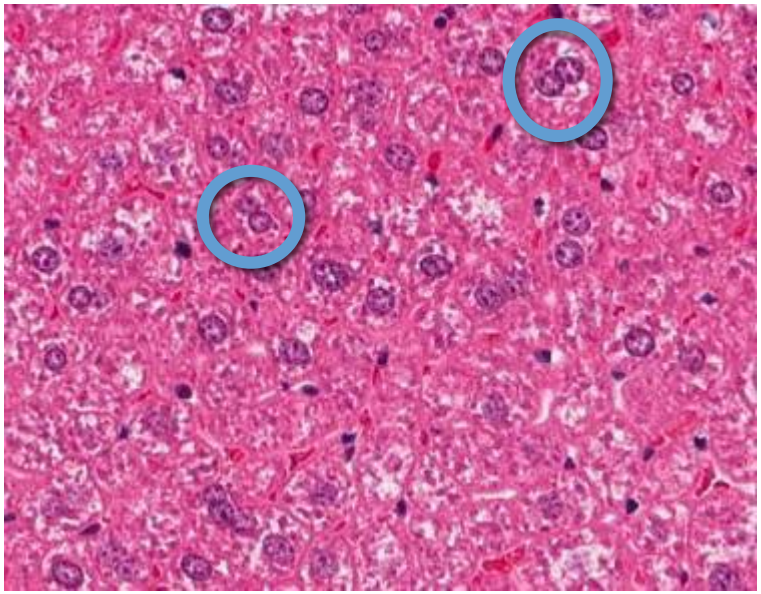


Cntrl n= 13, MMTV-Spy1 n= 12 *p-value = 0.000530852

Spy1 Increases Indices of Cell Division

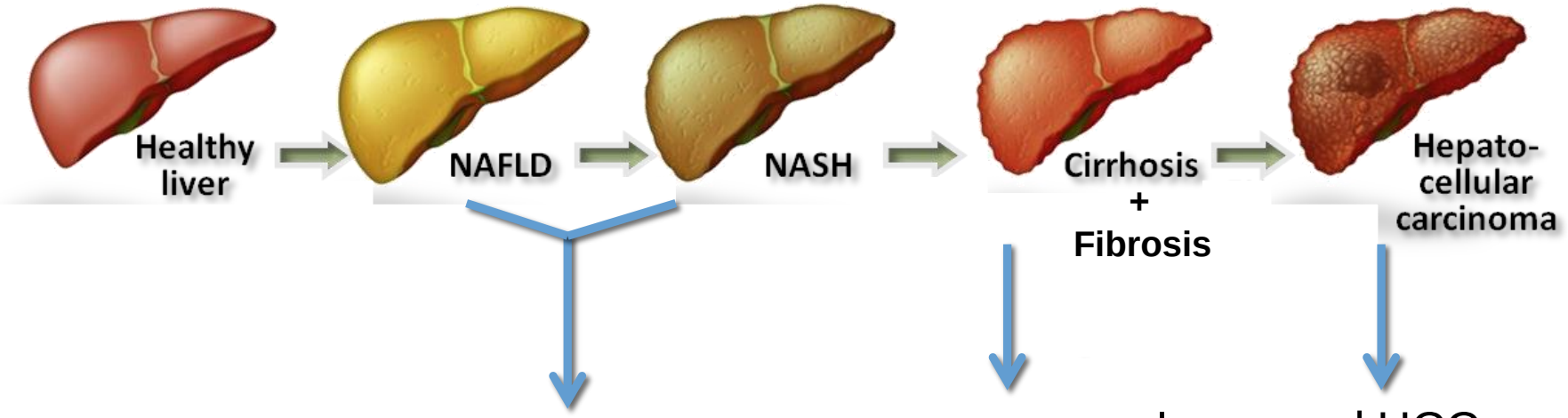
The percentage of bi-nucleated cells are significantly higher in control mice.





+1 yr control mouse

The Progression in MMTV-Spy1 Mice

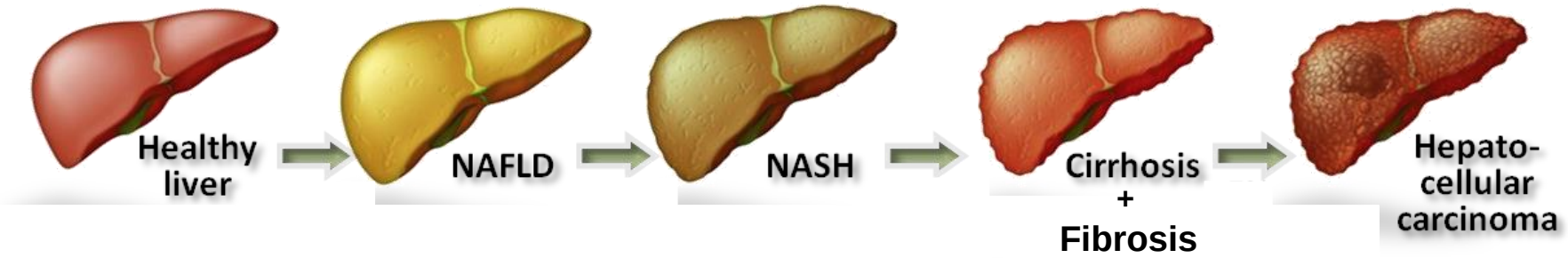


- Increased fat accumulation

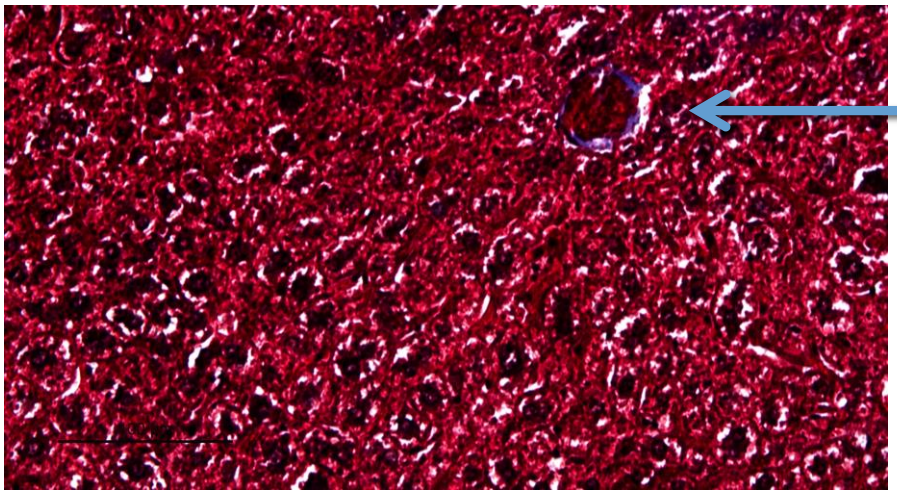
?

- Increased HCC
- Decreased bi-nucleated cells

The Progression in MMTV-Spy1 Mice



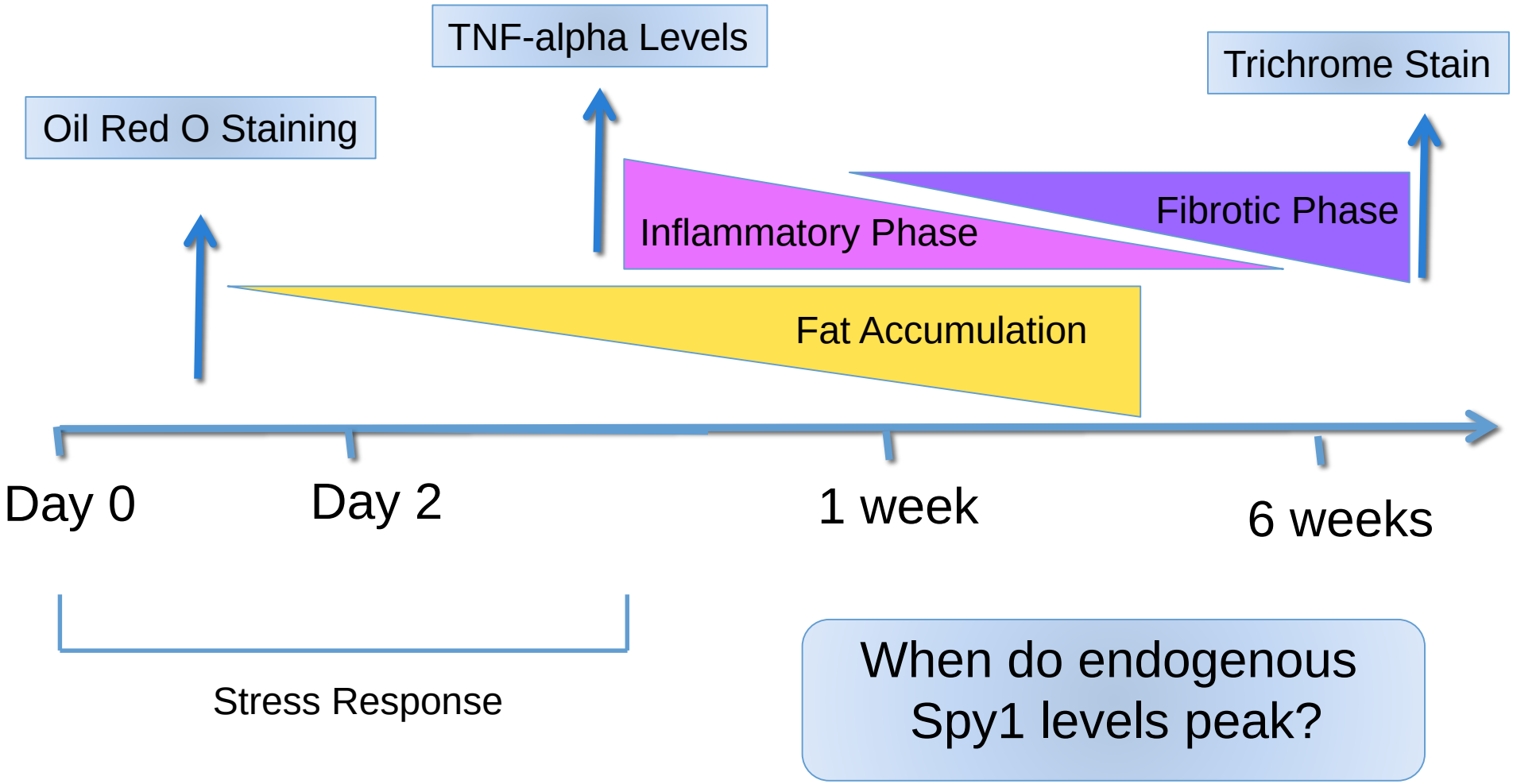
Trichrome stain of
+1yr MMTV-Spy1 mouse



The Methionine Choline Deficient (MCD) Diet

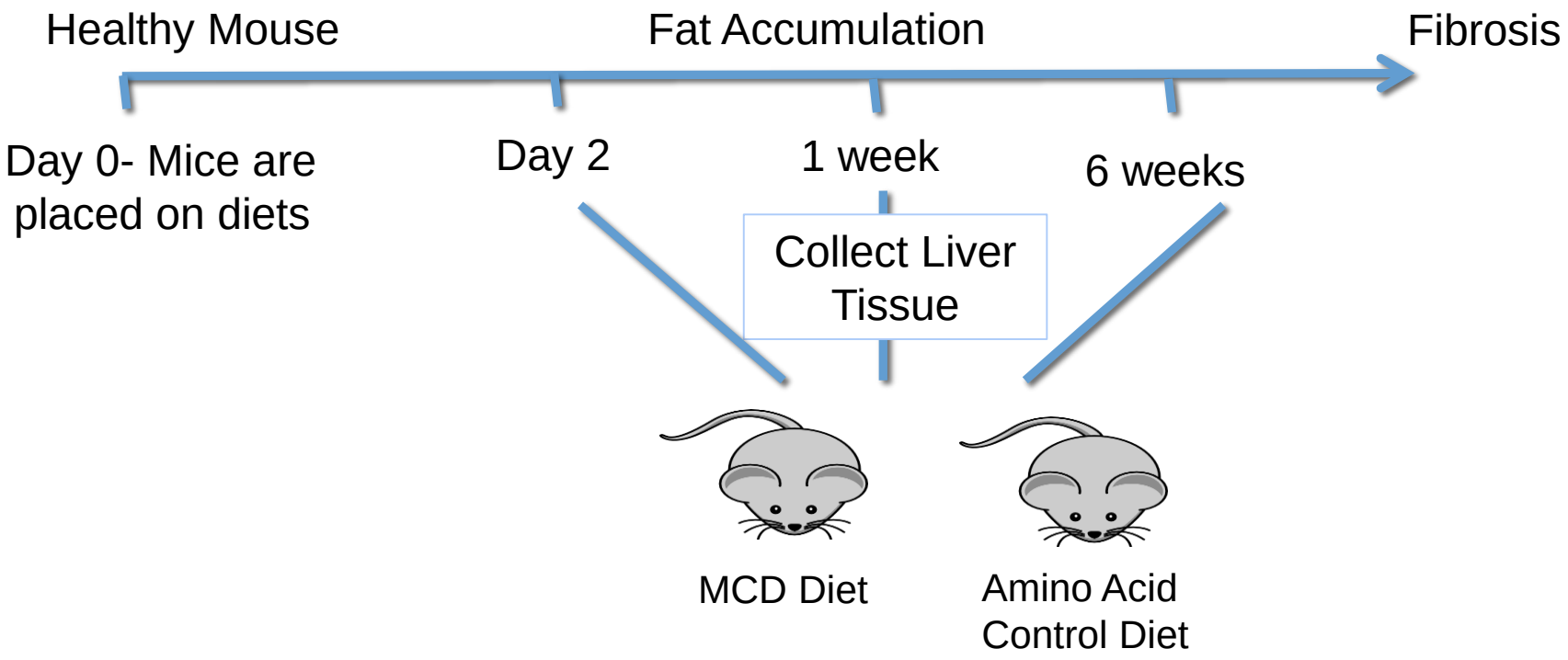
- Produces the most severe NASH phenotype in the shortest timeframe
- Causes increased fat accumulation in the hepatocytes
- Induces:
  - Inflammation
  - Apoptosis
  - Oxidative damage
  - Fibrosis
  - Increased serum alanine aminotransferase levels

MCD Progression



When do endogenous Spy1 levels peak?

The MCD Diet Experiment

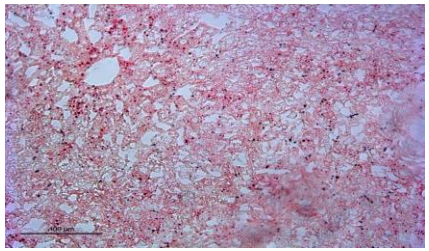


- Same genetic background as MMTV-Spy1 mice
  - Male mice between 8-12 weeks of age

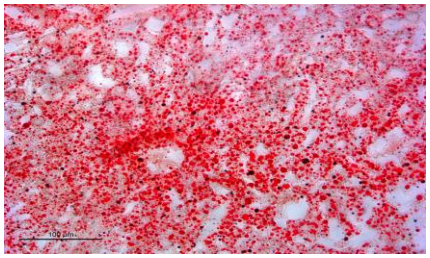
Tissue Collection and Analysis

- Formalin
  - H+E Staining
  - Immunohisto-chemistry
- Flash Frozen in Liquid Nitrogen
  - Analyze gene expression
  - Quantify protein levels
- 4% paraformaldehyde
  - Monitor fat accumulation

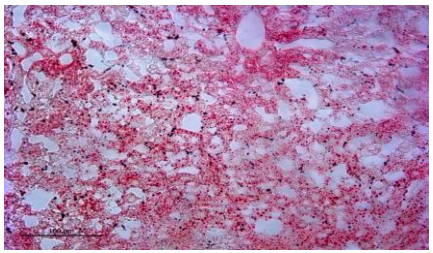
Fat Accumulation in MCD Mice



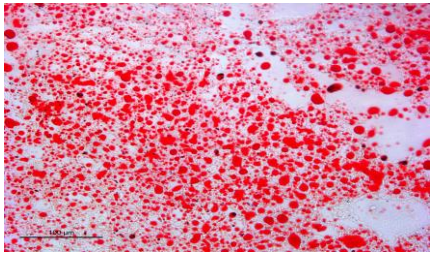
Day 2 control



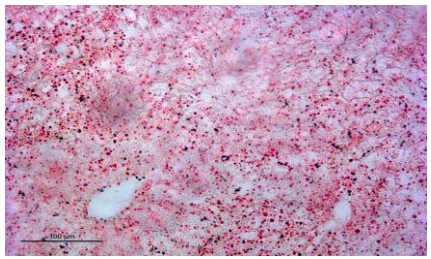
Day 2 MCD



1 week control

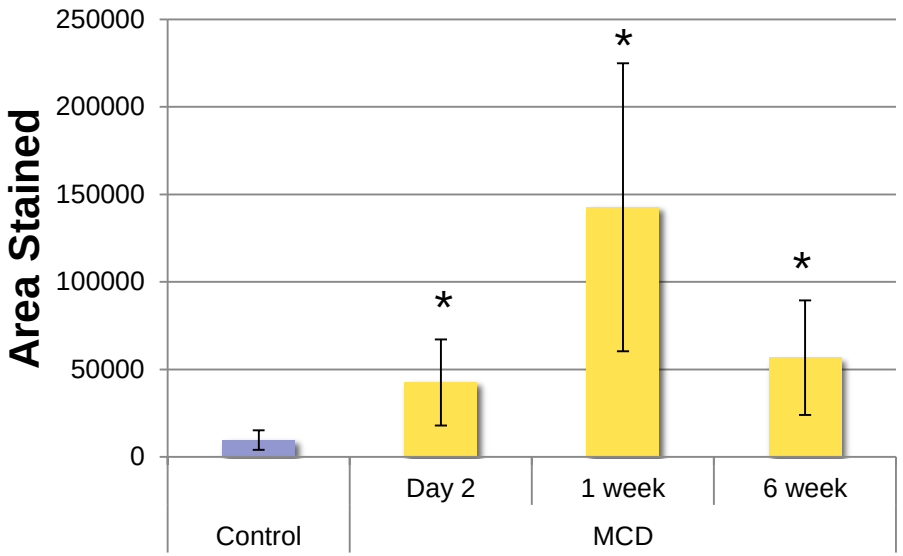


1 week MCD



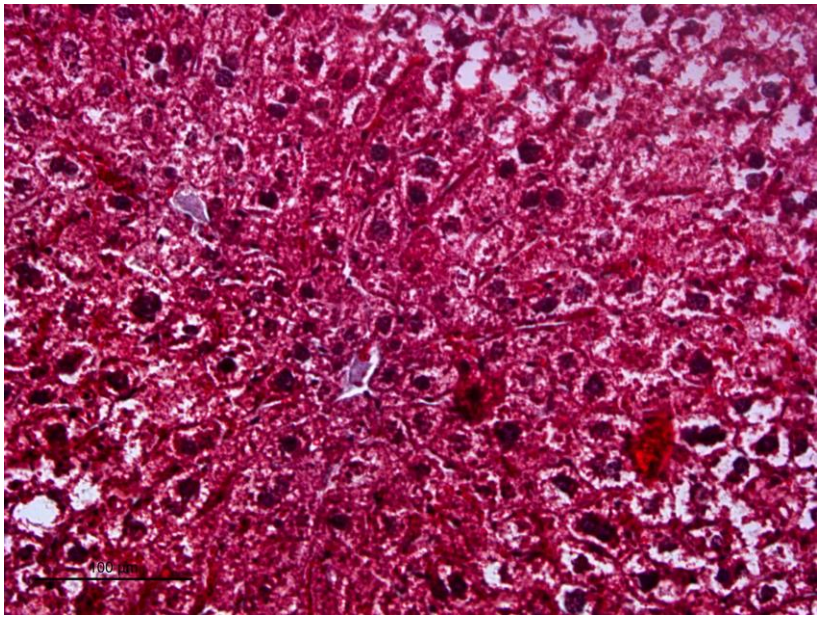
6 week control

6 week MCD

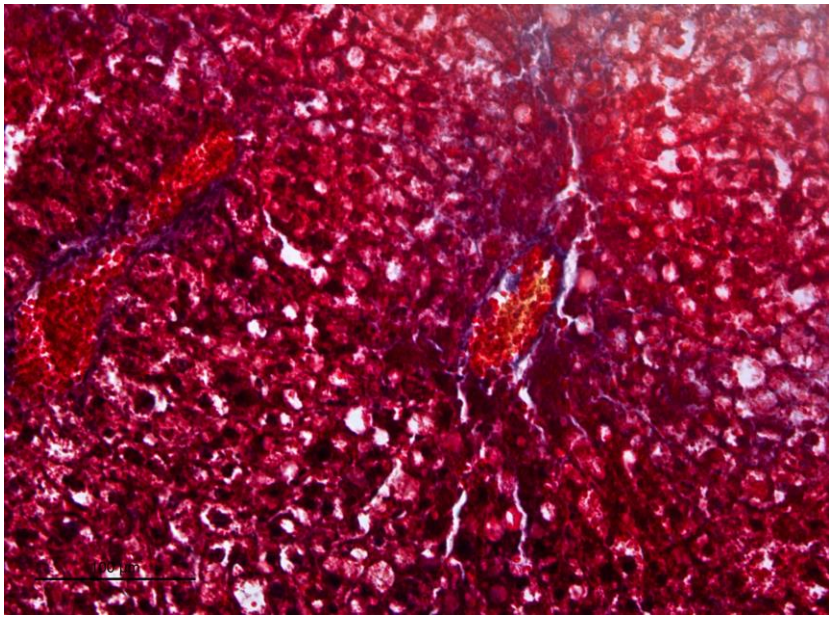


Fibrosis in MCD Mice

MCD mice had clear collagen deposition as compared to the controls.



6 week control



6 week MCD

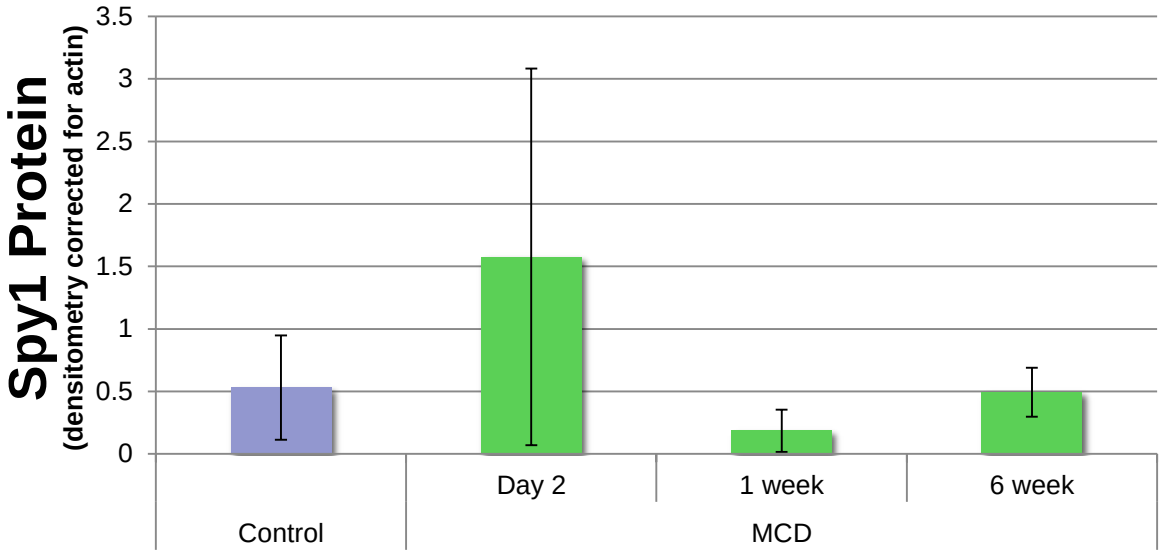
Spy 1 and p53 Levels

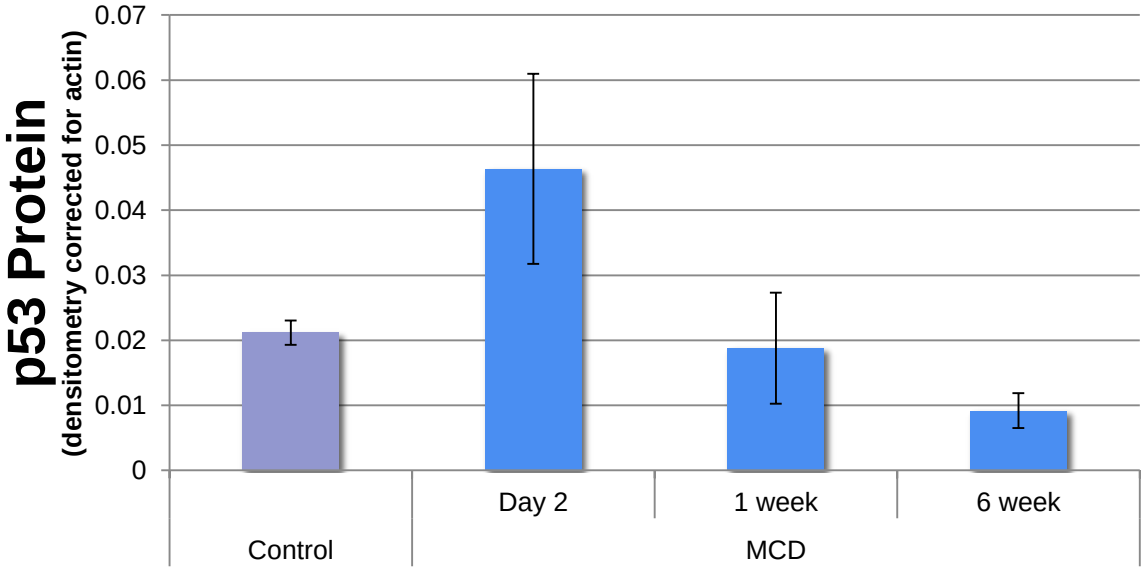
MCD Diet (NASH)

Activate p53

Halt the cell cycle

Apoptosis



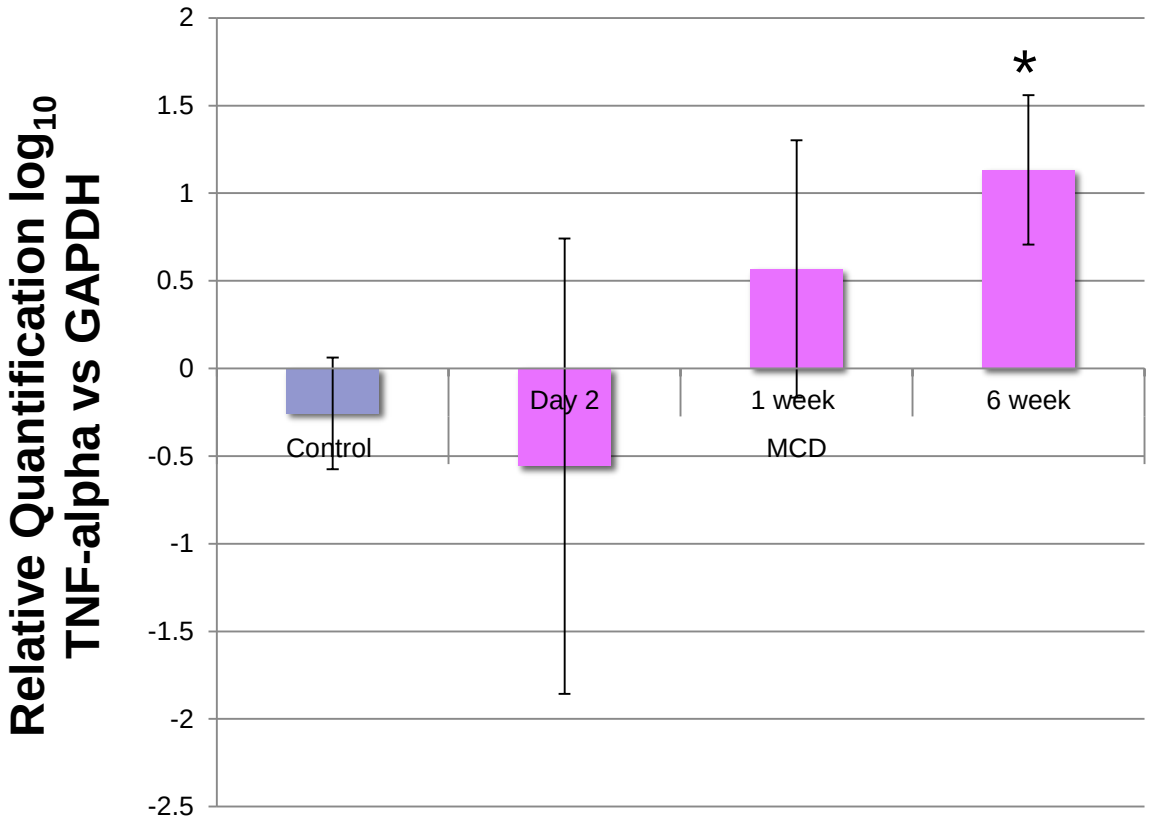


TNF-alpha Gene Expression

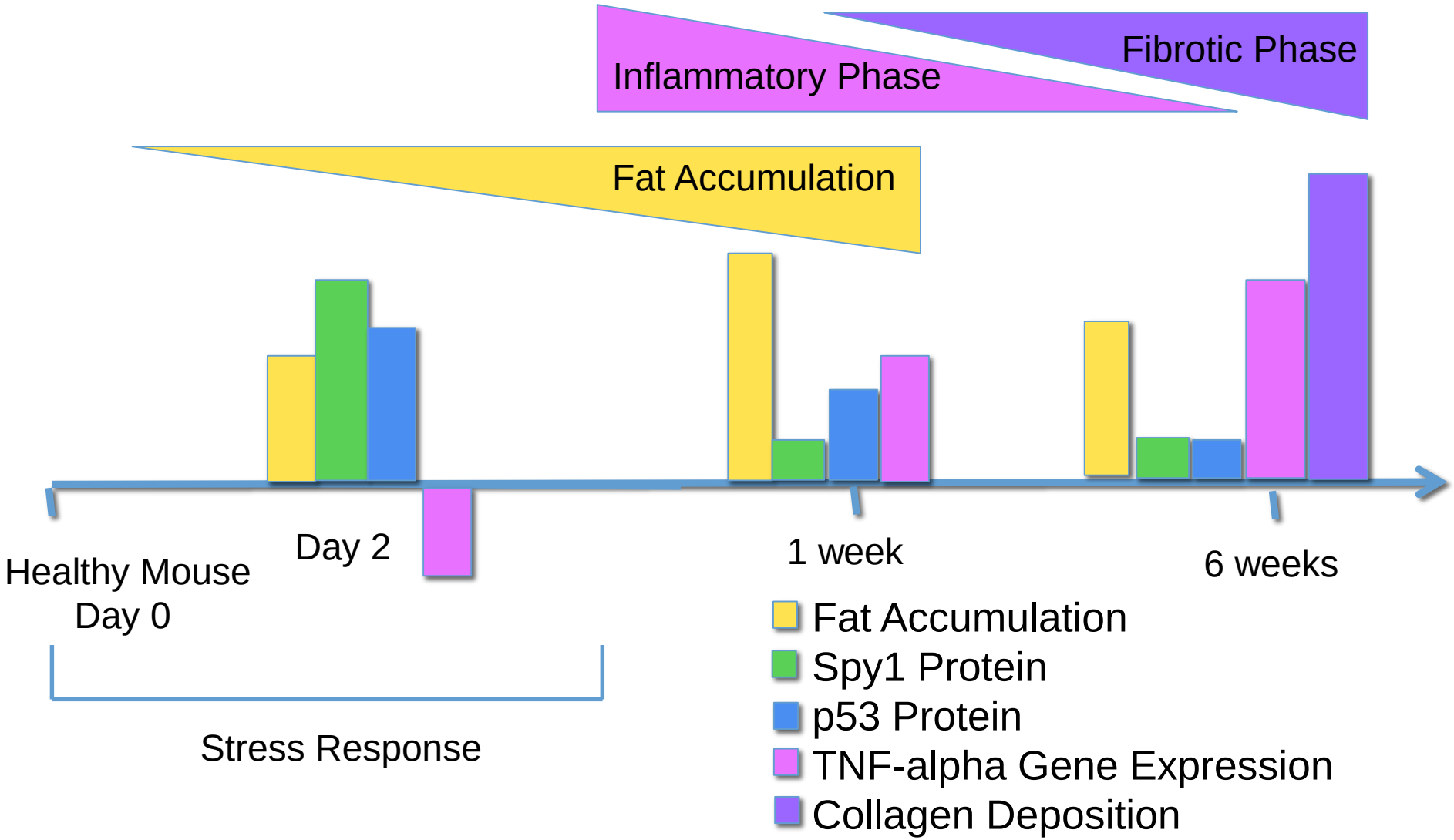
MCD Diet (NASH)

Inflammation

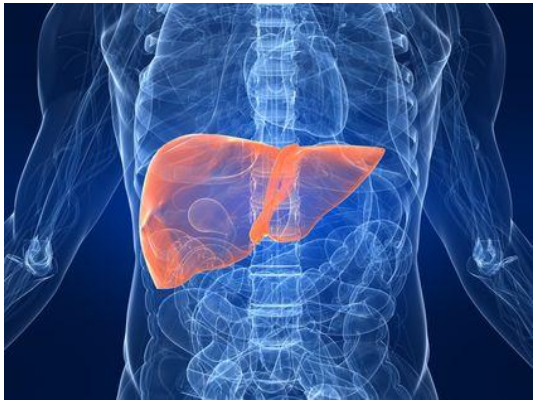
TNF-alpha activation



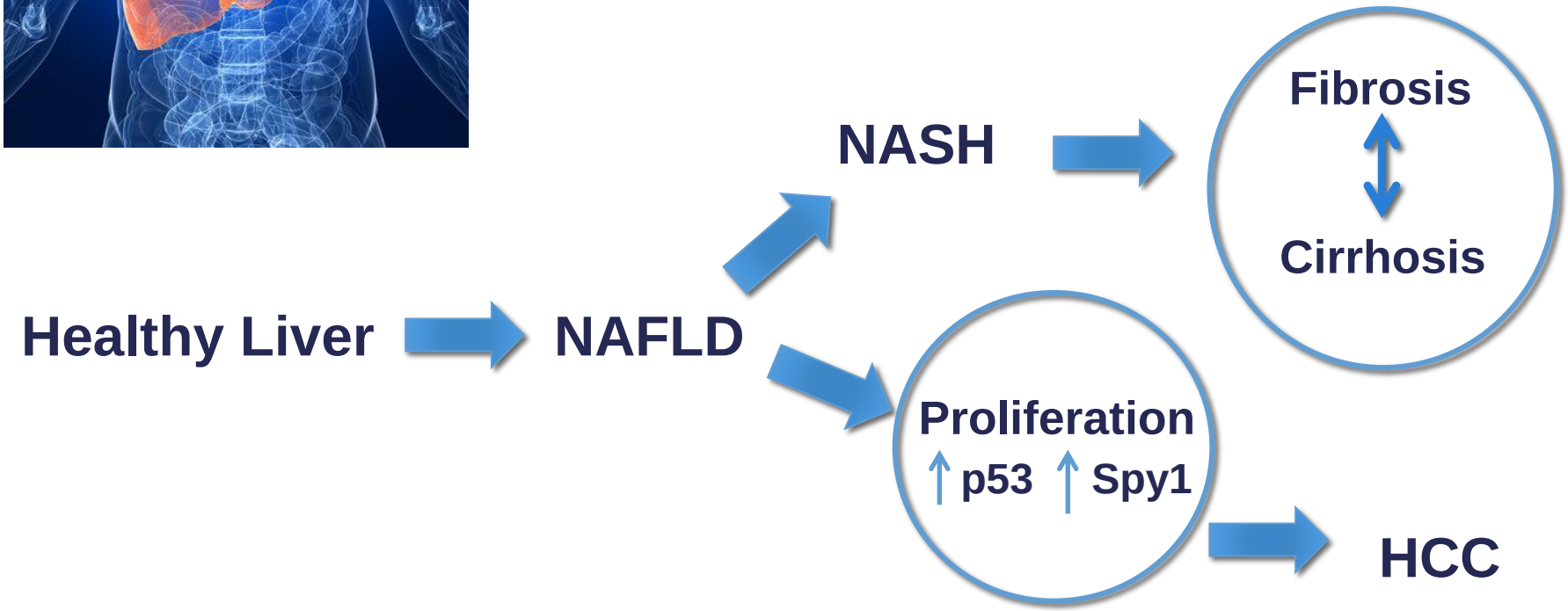
MCD Mice Progression



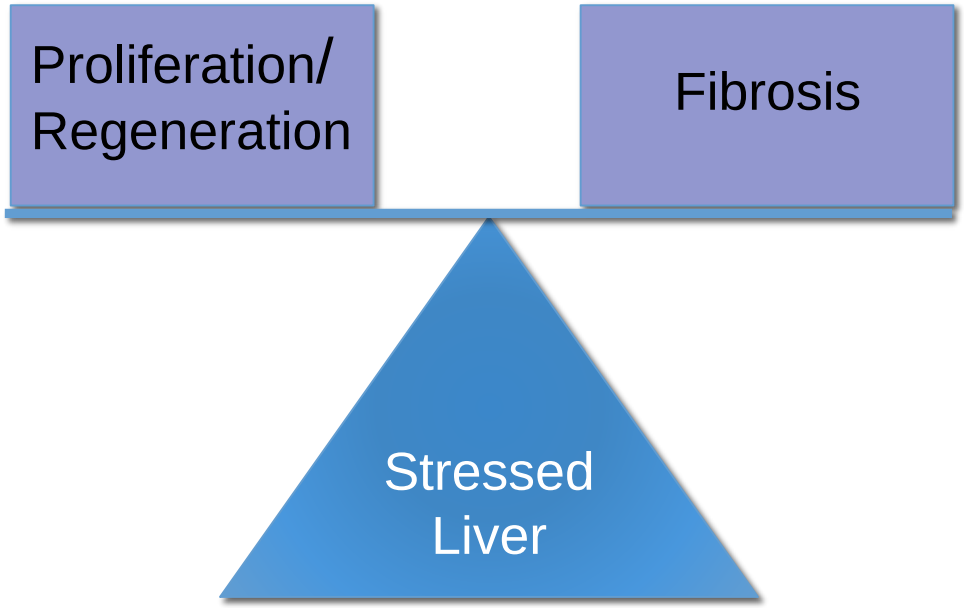
Revised Timeline of Progression



Spy1 as a prognostic indicator



Balance in the Face of Damage

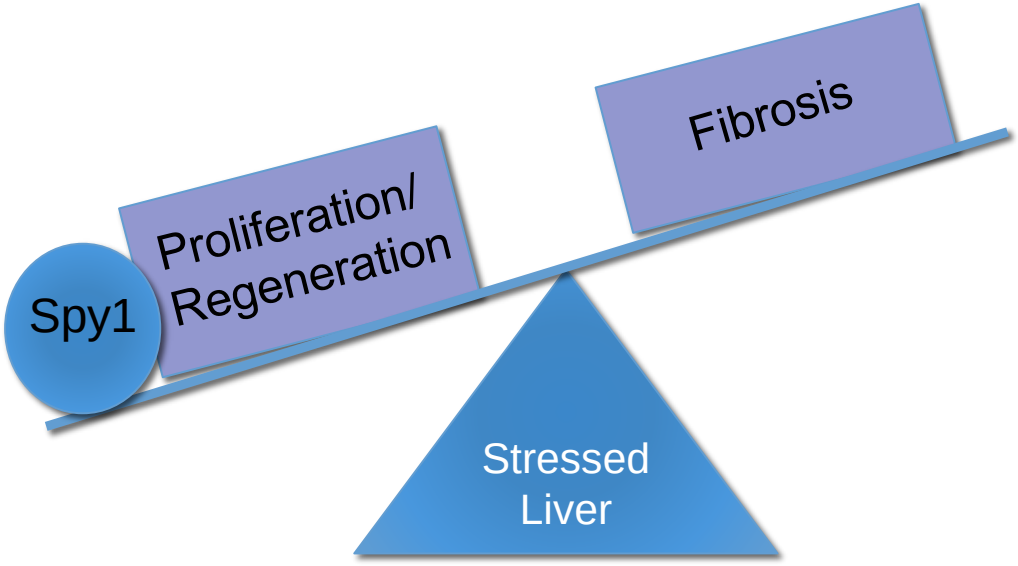


- Restores damaged hepatocytes
- Compensatory hyperplasia
- Allows for regeneration

- Maintains overall integrity of the organ
- Inflammation
- Formation of scar tissue
- Deposit collagen and fibrin

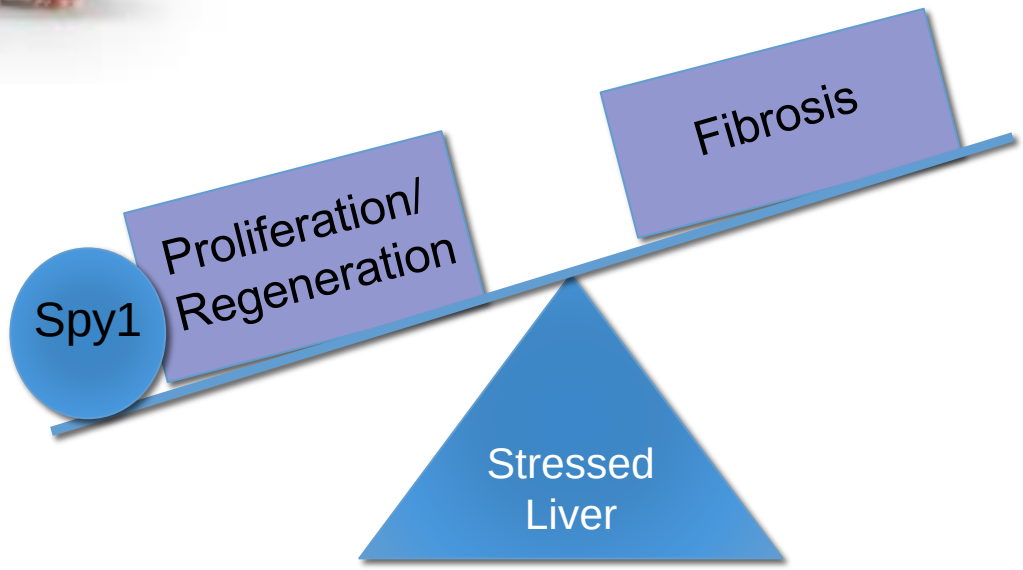
Does Proliferation Favor HCC Over Fibrosis ?

In response to an increase in fat accumulation and damage to hepatocytes, Spy1 will be up-regulated to increase regeneration and proliferative ability and decrease fibrosis.



Future Steps

Developing a Spy1 driven mouse
and follow it's progression on the MCD diet



Acknowledgements

Thank you to my wonderful supervisor, Dr. Lisa Porter and to my mentor Dr. Bre-Anne Fifield!

Thank you to the entire Porter Lab who have given me so much support and guidance!

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Seeds4Hope

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University of Windsor