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

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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

Diet/Lifestyle Factors

Hepatitis B/C

Alcoholism

Fatty Liver Disease

Steatohepatitis

Fibrosis

Cirrhosis

HCC

Healthy liver → NAFLD → NASH → Cirrhosis + Fibrosis → Hepatocellular carcinoma

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

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

B6CBAF1/J genetic background
HCC in the Spy1 Mouse Model

- MMTV-Spy1 male mice with high levels of Spy1 have significantly more HCC than their male littermate controls.
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
- Large vacuoles
- Disordered cell structure

Healthy liver +1yr control mouse
Fat accumulation +1yr MMTV-Spy1 mouse
HCC +1yr MMTV-Spy1 mouse
Fat Accumulation in MMTV-Spy1 Mice

10 month control mouse

10 month MMTV-Spy1 mouse

Oil Red O Stain Area

Control | MMTV-Spy1
---|---

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

Healthy liver → NAFLD → NASH → Cirrhosis + Fibrosis → Hepatocellular carcinoma

Trichrome stain of +1yr MMTV-Spy1 mouse

Collagen
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

- **Stress Response**
- **TNF-alpha Levels**
- **Inflammatory Phase**
- **Fat Accumulation**
- **Fibrotic Phase**
- **Trichrome Stain**

**When do endogenous Spy1 levels peak?**
The MCD Diet Experiment

Day 0 - Mice are placed on diets

Day 2

1 week

6 weeks

Collect Liver Tissue

MCD Diet

Amino Acid Control Diet

- Same genetic background as MMTV-Spy1 mice
- Male mice between 8-12 weeks of age
Tissue Collection and Analysis

Formalin

4% paraformaldehyde

Flash Frozen in Liquid Nitrogen

H+E Staining

Immunohistochemistry

Analyze gene expression

Quantify protein levels

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

Area Stained

Control  |  Day 2  |  1 week MCD  |  6 week MCD

*
Fibrosis in MCD Mice

MCD mice had clear collagen deposition as compared to the controls.
Spy 1 and p53 Levels

MCD Diet (NASH) → Activate p53 → Halt the cell cycle → Apoptosis

**Graph 1:** Spy1 Protein
- Control
- Day 2
- 1 week MCD
- 6 week

**Graph 2:** p53 Protein
- Control
- Day 2
- 1 week MCD
- 6 week

(Densitometry corrected for actin)
TNF-alpha Gene Expression

MCD Diet (NASH) → Inflammation → TNF-alpha activation

Relative Quantification $\log_{10}$ TNF-alpha vs GAPDH

- Control
- Day 2
- 1 week
- 6 week

* indicates statistically significant difference.
MCD Mice Progression

- **Healthy Mouse Day 0**
- **Day 2**
- **1 week**
- **6 weeks**

- **Fat Accumulation**
- **Spy1 Protein**
- **p53 Protein**
- **TNF-alpha Gene Expression**
- **Collagen Deposition**

**Phases:**
- Inflammatory Phase
- Fibrotic Phase

**Stress Response**
Revised Timeline of Progression

Healthy Liver → NAFLD → NASH → Proliferation → Fibrosis/Cirrhosis → HCC

Spy1 as a prognostic indicator

↑ p53  ↑ Spy1
Balance in the Face of Damage

- Proliferation/Regeneration
  - Restores damaged hepatocytes
  - Compensatory hyperplasia
  - Allows for regeneration

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

Stressed Liver
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 its progression on the MCD diet
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