Cytokinesis inhibition in the liver drives polyploidization and HCC prevention

Hao Zhu, MD
October 17, 2020
Conflicts of interest

I consult for 28-7 Therapeutics

I collaborate with Alnylam Therapeutics

I own stock in Ionis
Chronic liver disease from any cause ultimately results in cirrhosis.
We dissect the cellular + genetic events culminating in cirrhosis, liver cancer.
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1. Genetic/mutational events?

2. Cellular fates?

Chronic injury → Cell death/damage → Improved fitness/function

Hepatocellular carcinoma
Hypothesis:

Polyploidy in hepatocytes allows the liver to safely sustain mutagenesis (w/o carcinogenesis) during wound healing.
Up to 90% of mouse and 50% of human hepatocytes are polyploid


Liver diseases involve chronic injury, eventually leading to HCC.
How does chronic injury affect the ploidy in normal liver?

- Chronic injury
- Cell death and damage
- Regeneration

Inflammation and Fibrosis → Liver Cancer (HCC)
Chronic injury increases hepatic polyploidy

Chronic Carbon tetrachloride (CCl₄) injury

Wildtype mice

Ctrl

CCl₄

Rosa

Diploid  Tetraploid  Octaploid

2c  4c  8c

Ctrl  CCl₄  Ctrl  CCl₄  Ctrl  CCl₄

Percentage

2c  4c  8c

**P=0.003  ****P<0.0001  ****P<0.0001

Ctrl  CCl₄  Ctrl  CCl₄  Ctrl  CCl₄

Percentage
A genetic switch to study ploidy: ANLN (Anillin) cytokinesis protein


Green RA, et al., Cytokinesis in animal cells[Annual review of cell and developmental biology, 2012

Inducible *TG-shAnln* mice are a tool to increase polyploidy

Zhang S et al., *Dev Cell*, 2018

![Diagram showing the expression of *shAnln* and *GFP* with RNA interference under Dox induction.](image)

**Ploidy distribution (P28)**

- **Rosa**
- **TG-shAnln**

**Percentage**

- 8c
- 4c
- 2c

![Graph showing ploidy distribution with statistical significance](image)

**Percentage**

- 0%
- 20%
- 40%
- 60%
- 80%
- 100%

- **Rosa**
- **TG-shAnln**

- **ns**
- ****** P<0.0001**

Zhang S et al., *Dev Cell*, 2018
Knocking down ANLN in mice increases polyploidy.
Does increased ploidy influence chronic injury-induced HCC?
Polyploidy prevents chronic injury-induced HCC development

Control or Super-polyploid

Rosa or TG-shAnln

ON  Dox  OFF
P0  P20

DEN
P35 (75μg/g)
P38  Twice/week 12 weeks

Assess tumor burden at P160

Rosa  TG-shAnln

Tumor quantification

Surface Tumor number/liver

Rosa  TG-shAnln

Nodule quantification

Tumor nodule number/slide

Rosa  TG-shAnln

2.5 mm
Diploidy increases chronic injury-induced HCC development
Does polyploidy influence pathogenic steps to cancer?

Chronic injury → Cell death and damage → Regeneration

Inflammation and Fibrosis → HCC

Polyploid HCC
Does polyploidy influence pathogenic steps to cancer?

**Chronic injury** → **Cell death and damage** → **Regeneration**

**Inflammation and Fibrosis** → **HCC**

*Polyploid HCC*
Can polyploid cells proliferate and regenerate the liver?

- Chronic injury
- Cell death and damage
- Regeneration
  - Proliferate less
  - Tricky mitosis
- Inflammation and Fibrosis
- HCC
- Polyploid HCC
Polyploid cells can regenerate the liver after acute injury

Control or Super-polyploid

Rosa or TG-shAnln

Dox 70% PHx

P0 P20 P42

48 hour harvest before after

LW/BW

Rosa TG-shAnln

Rosa TG-shAnln

p-H3+ percentage

15 10 5 0

Rosa TG-shAnln
Polyploidy does not affect gene expression in regeneration

RNA-seq

During regeneration

Super-polyploid

Control
Polyploid hepatocytes readily divide, with high fidelity

Mitosis staining in the regenerating liver

- Diploid
- Polyploid
- Super-polyploid

**Control**

- Rosa
- 8c 8c
- 4c 4c

**TG-shAnln**

- 8c 16c
- 4c 4c

**Prometaphase**

- Control
- Super-polyploid

**Metaphase**

- αTubulin

**Anaphase**

- γTubulin

- Hoechst
In human livers, aneuploid nodules are rare.

Whole-exome seq

54 Individual nodules

Chr 1 & Chr 8 aneuploidy (Positive control) Zhu M et al., Cell, 2019
Tumor suppressor loss of heterozygosity reduced in polyploids
Polyploid hepatocytes protect from cancer while maintaining regenerative capacity in chronic liver disease.

Regenerative capacity does not cause chromosome missegregation and aneuploidy. Polyploidy could be an adaptation to buffer against TSG mutations.

How do polyploids ensure segregational fidelity during mitosis?
Cytokinesis inhibition and polyploidization with **ANLN** siRNAs for HCC prevention?

Cirrhotic patients with higher risk for HCC

**GalNAc-siRNA**

PROS of hepatocyte-specific siRNA:
- Achievable, FDA approved
- Less toxic than systemic small molecules

**Transient inhibition: Ploidy**

**Continuous inhibition**

Ploidy change

Cancer initiation

Analysis

Inhibition

Inhibition/deletion

Cancer initiation
Persistent ANLN shRNA inhibition: DEN mutagen HCC model

Initiation with DEN

P15

Knockdown every other week

shRNA

shRNA

shRNA

shRNA

P126

Analysis

P180

Intermittent ANLN inhibition

Tumor quantification

*** P < 0.001
Persistent ANLN shRNA inhibition: chronic DEN + CCl4 injury
Floxed ANLN genetic models

ANLN knockout livers appear grossly normal, regenerate after hepatectomy, and are highly polyploid.
Assessing ANLN knockout efficiency using AAV-Cre in NASH

AAV-Cre effect disappears in 3 months.
Second dose has no effect (possibly due to immunogenicity)
Despite poor KO at 3 months, NASH related HCC is suppressed at 9 months of age
ANLN KO via AAV-Cre prevents liver damage in NASH model
ANLN KO via AAV-Cre prevents steatosis in NASH model

N = 5 and 5 mice shown here
ANLN KO via AAV-Cre suppresses fibrogenesis in NASH
Can this be replicated with Alnylam siRNAs?
Knockdown efficiencies of GalNAc conjugated siAnlns

**in vitro:**

**in vivo:**
GalNAc siAnln did not affect acute or chronic tissue repair

Hepatectomy

CCI4

hepatocyte transplant

Day -2: Transplantation
Day 0: Start treatment

Body weight ratio to Day 0

Days after siRNA treatment started
Multiple HCC models are prevented by GalNAc-siANLN
Summary: safe, and effective for HCC prevention

Prospective clinical use:
Patient with cirrhosis and small Lirads/hcc lesion <2 cm.
Patient with early HCC resected or ablated.
Acknowledgements

Clinical collaborators:
Amit Singal
Adam Yopp
David Hsieh
Nicole Rich
Yujin Hoshida