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The Investigation of Virus-Driven Intrahepatic Cholangiocarcinoma through Dysregulated Hepatic Differentiation

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Introduction



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HCV as a risk factor for iCCA

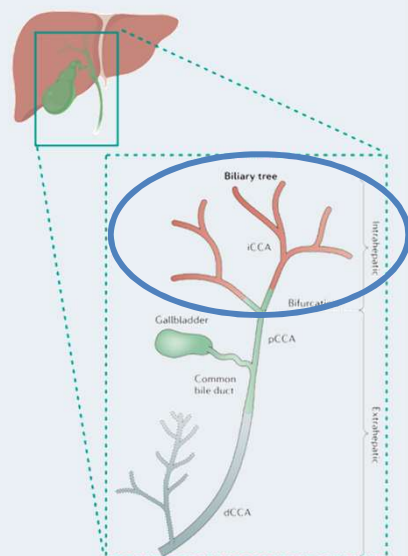


Plasticity within the hepatic compartment and possible cell of origin of HCV driven iCCA

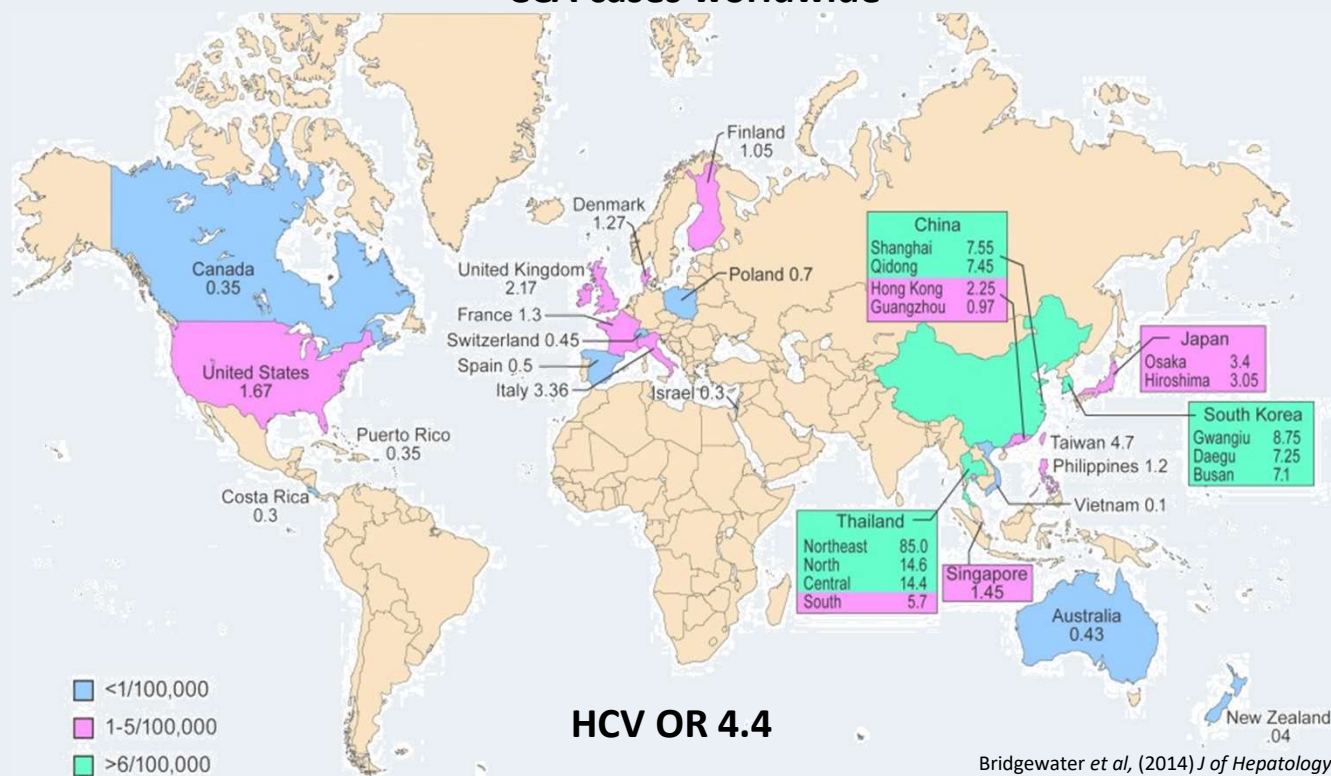


Pathway that may be involved

Hepatitis C virus as a risk factor for iCCA



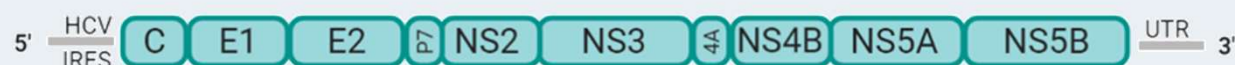
CCA cases worldwide



↑iCCA in the westernised world → could this be associated with epidemiological pattern of chronic liver diseases such as viral hepatitis?

Hepatitis C virus as a risk factor for iCCA

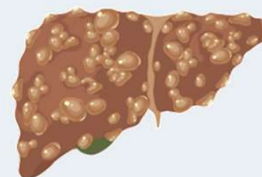
Hepatitis C Virus Genome



HCV Class A Carcinogen

HCV treated with
DAAs = 90% 'clinically
cured'

≠↓PLC risk unlike
previous interferon
based treatments



Indirect
-Inflammation



Direct
-Viral oncogenes
-'hit and run'

HCV and Epigenetic Imprinting

Hamdane *et al*, (2019) *Gastro*

HCV-Induced Epigenetic Changes Associated With Liver Cancer Risk Persist After Sustained Virologic Response

Perez *et al*, (2019) *PLoS One Gen*

Hepatitis C virus leaves an epigenetic signature post cure of infection by direct-acting antivirals

Demonstrate epigenetic changes as a result of HCV associated with HCC risk persisting post cure

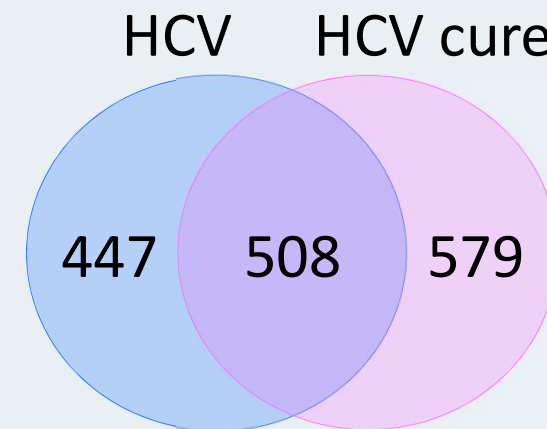
HCV and Epigenetic Imprinting

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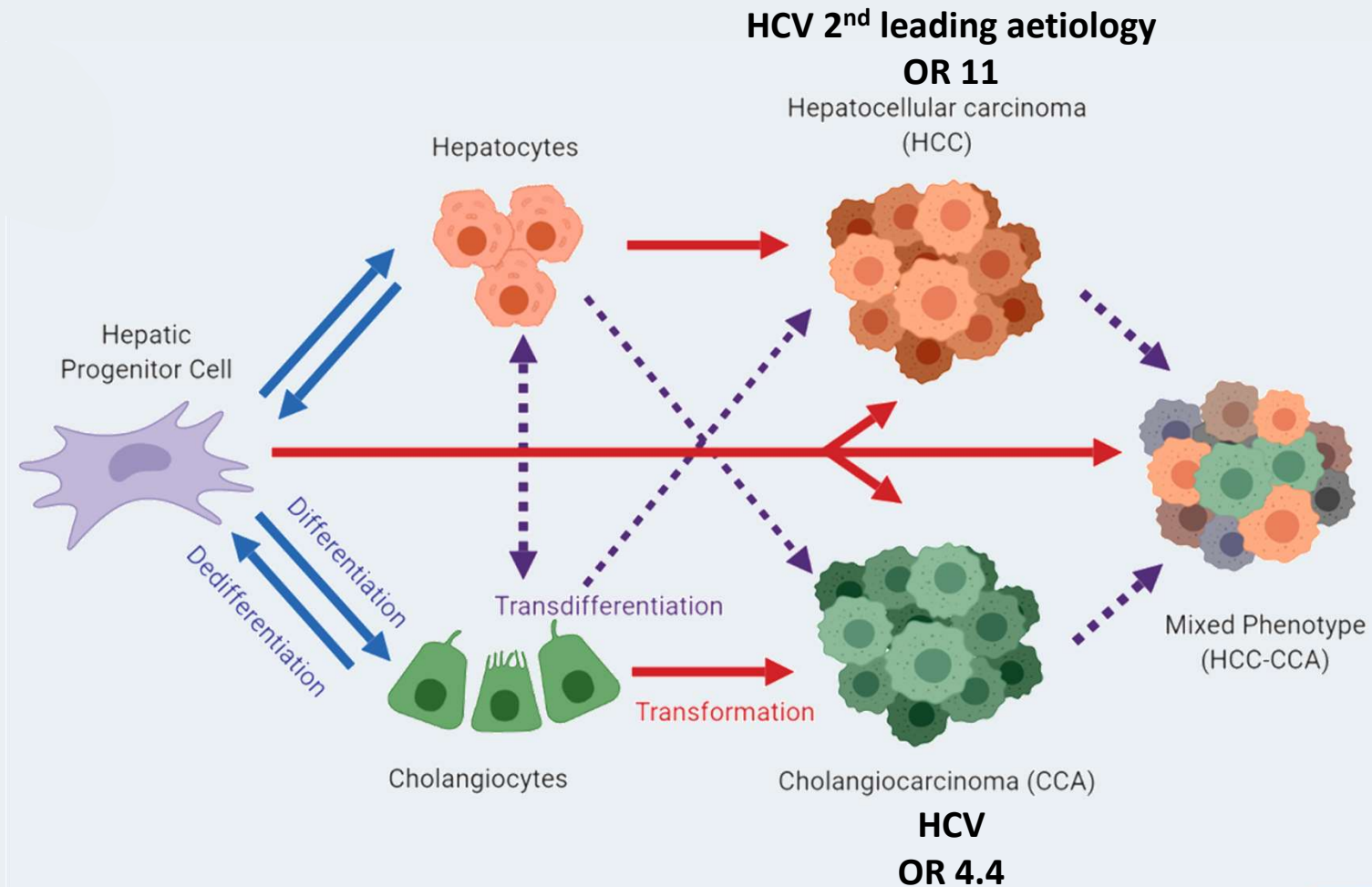


Demonstrate epigenetic changes as a result of HCV associated with HCC risk persisting post cure

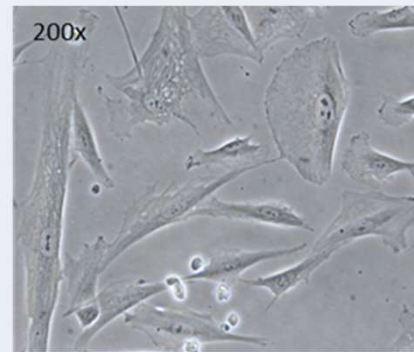
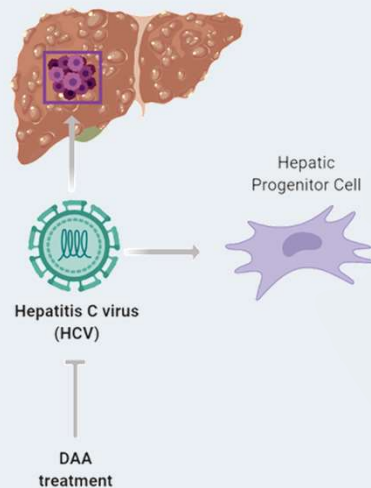
High Plasticity within the Hepatic Compartment



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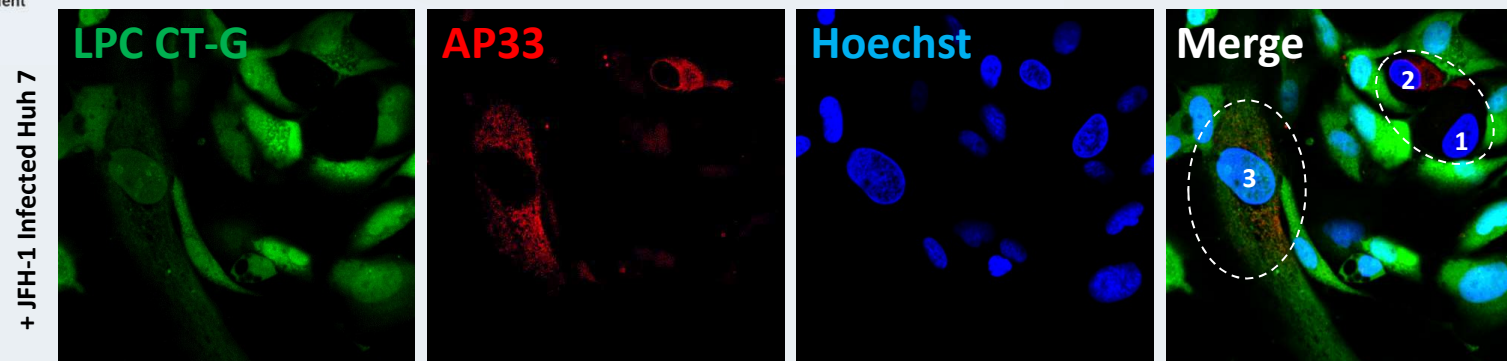
Can HCV infect HPCs?



Ex vivo human derived HPCs

**HCV can infect iPSC derived HPCs
and ex vivo HPCs**

→ Could HPCs be the origin of HCV
driven iCCA?

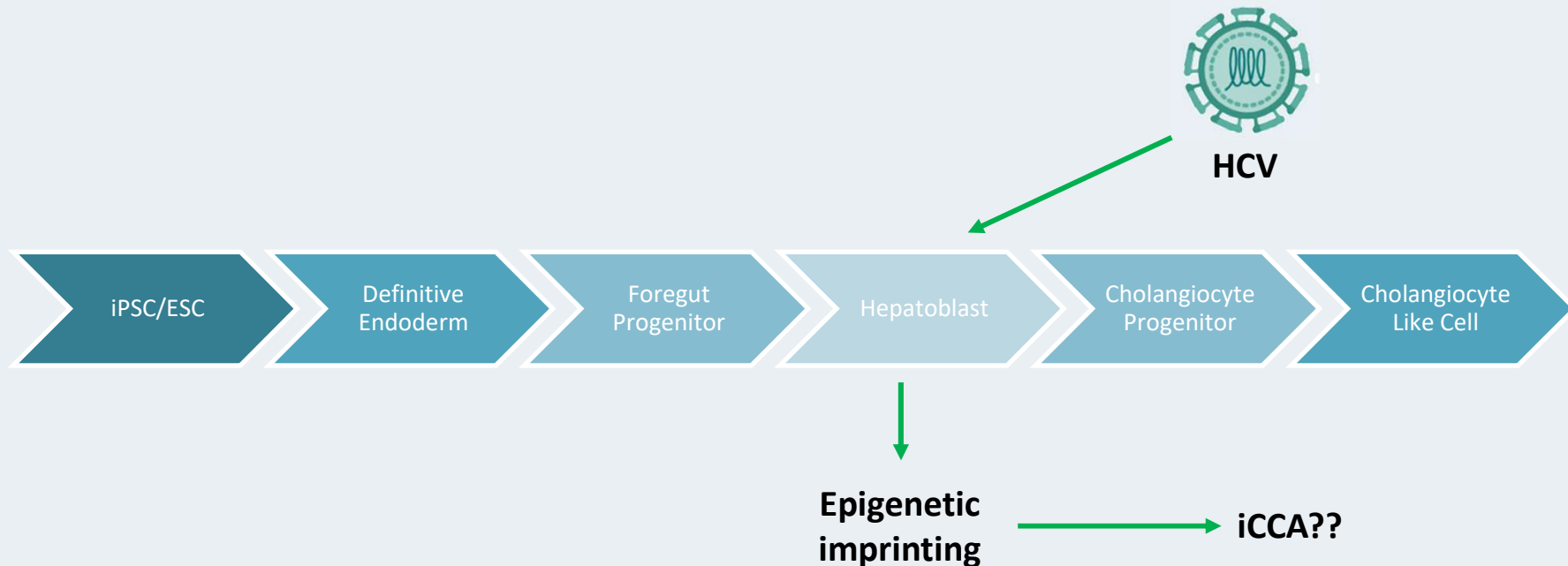


Co-culture with control/HCV-infected Huh7 cells – 1: Uninfected Huh7 cell; 2: Infected Huh7 cell; 3: Infected LPC

Developing a stem cell derived model

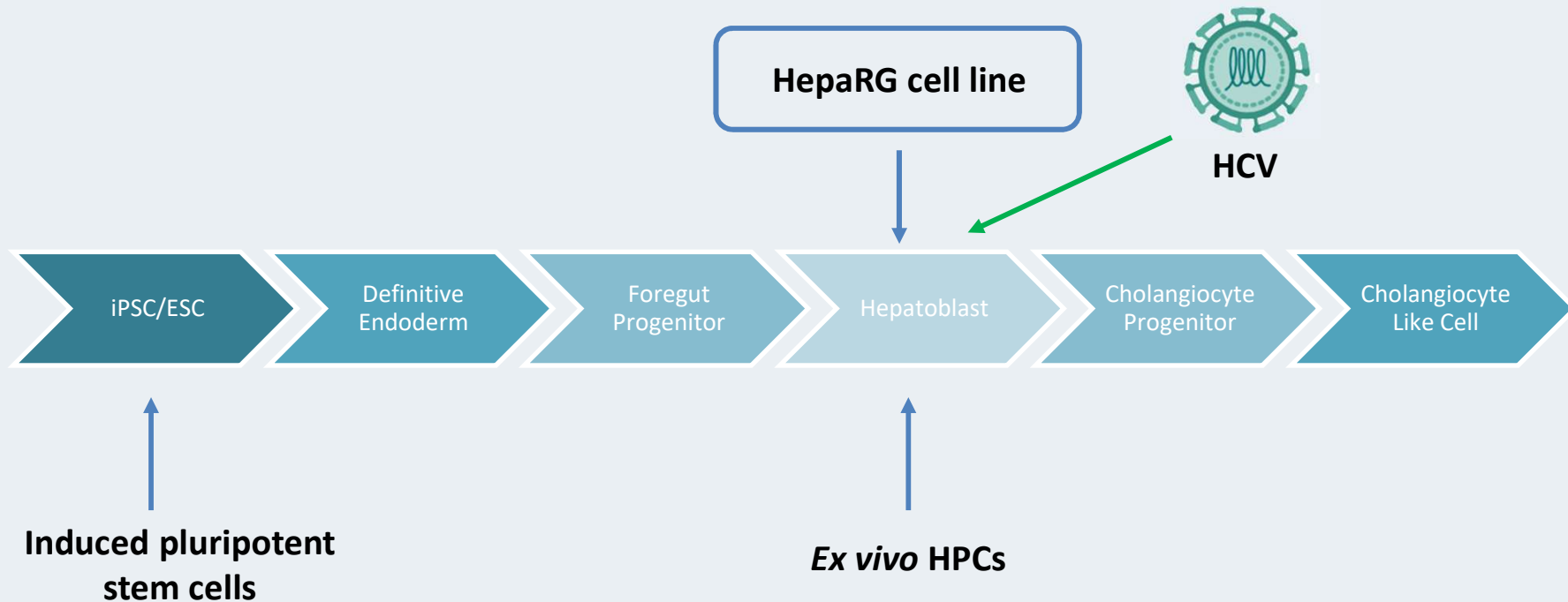


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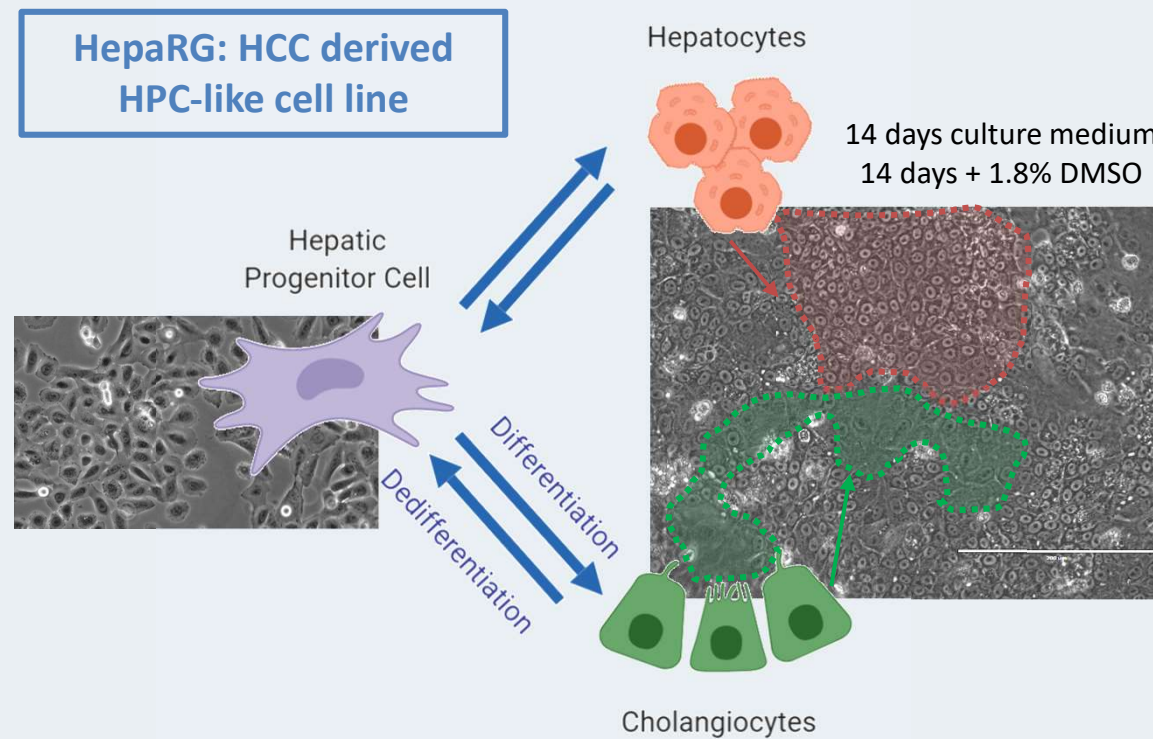
Hypothesis: HCV infection results in an imprinted pro-tumourigenic transcriptional phenotype that persists following DAA treatment

Developing a stem cell derived model



1. Can HCV infect our cell line of choice?
2. Can this cell line be differentiated towards a cholangiocyte specific population?

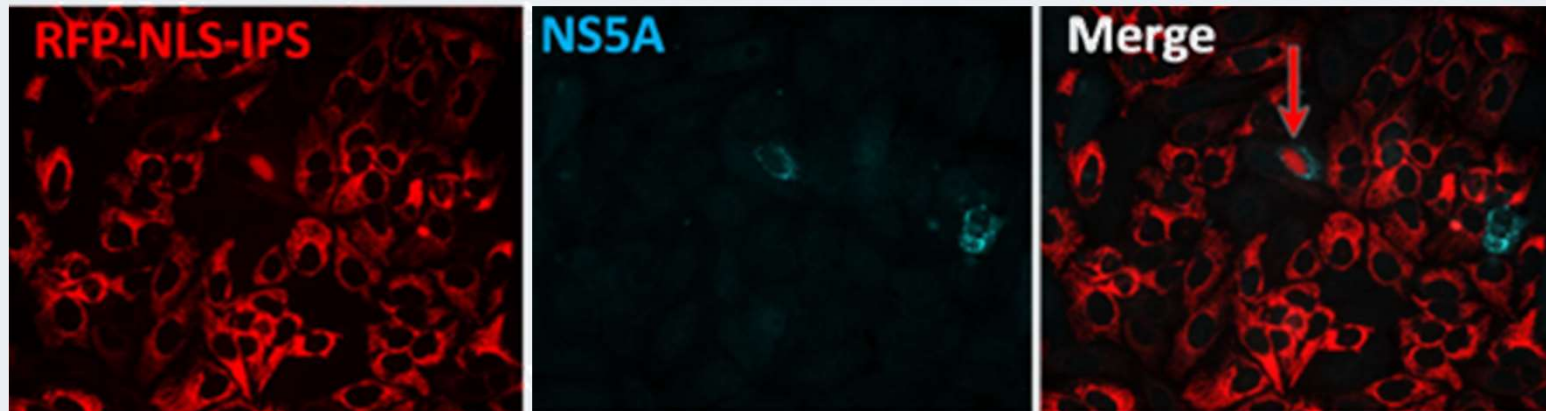
HepaRG cell line



Parent *et al*, (2004) *Gastroenterology*
Cerec *et al*, (2007) *Hepatology*

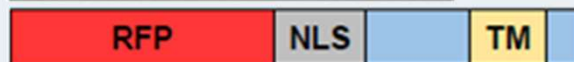
HepaRG HCV infection

1. Are HepaRG cells permissive to HCV?



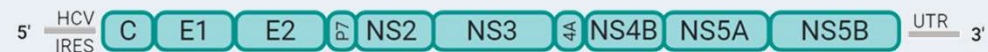
J6-JFH1 HCV + HepaRG RFP-NLS-IPS-1 cells

RFP-NLS-IPS-1 lenti-construct



Cleaved by NS3-4A
RFP → Localised to
nucleus

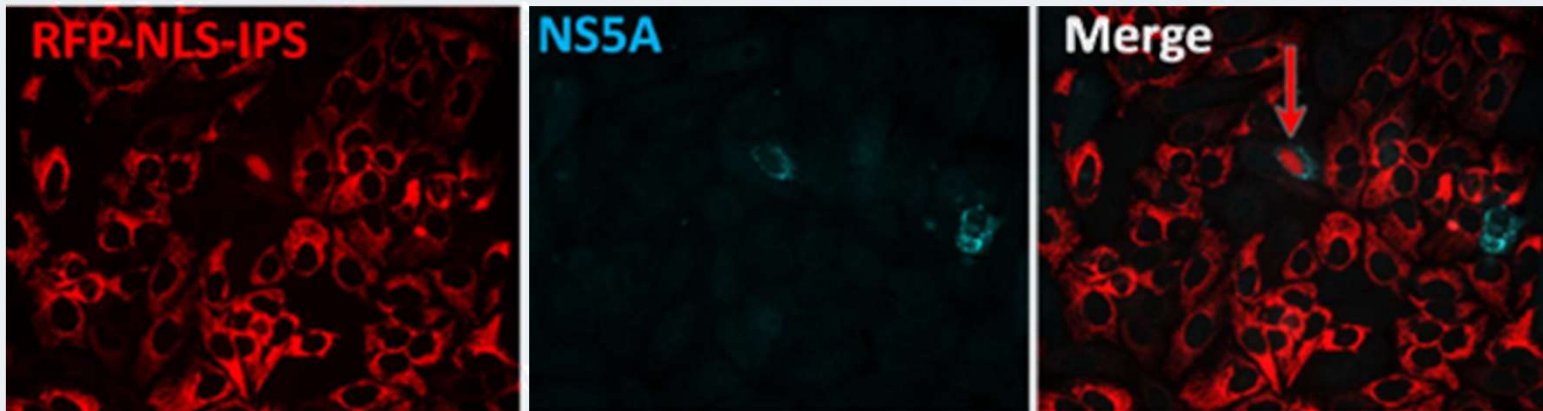
Hepatitis C Virus Genome



Full length virus

HepaRG HCV infection

1. Are HepaRG cells permissive to HCV?



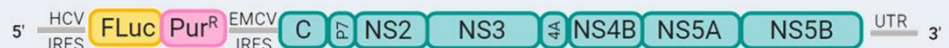
J6-JFH1 HCV + HepaRG cells

Currently optimising for transfection
with HCV subgenomic replicons →
puromycin selection
luciferase assay detection

Hepatitis C Virus Genome



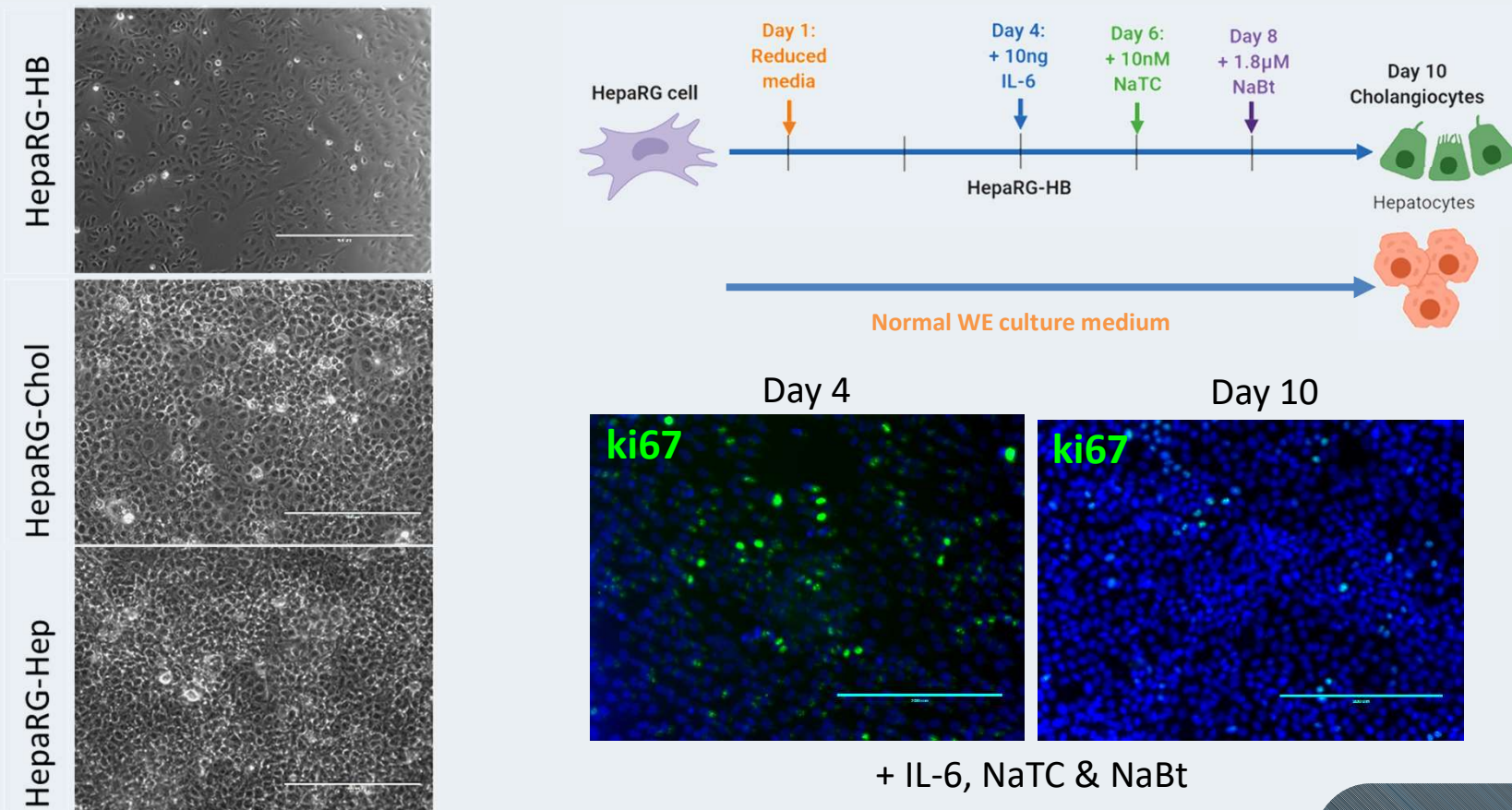
N17/JFH1 subgenomic replicon



Cholangiocyte differentiation

2. Can HepaRG cells be differentiated towards a cholangiocyte-like cell specific population?

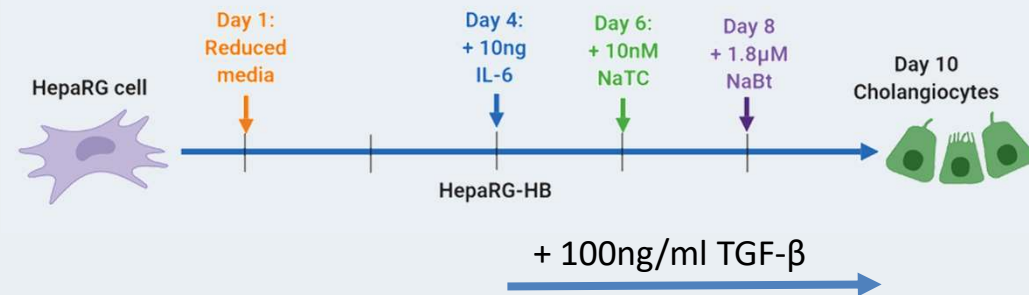
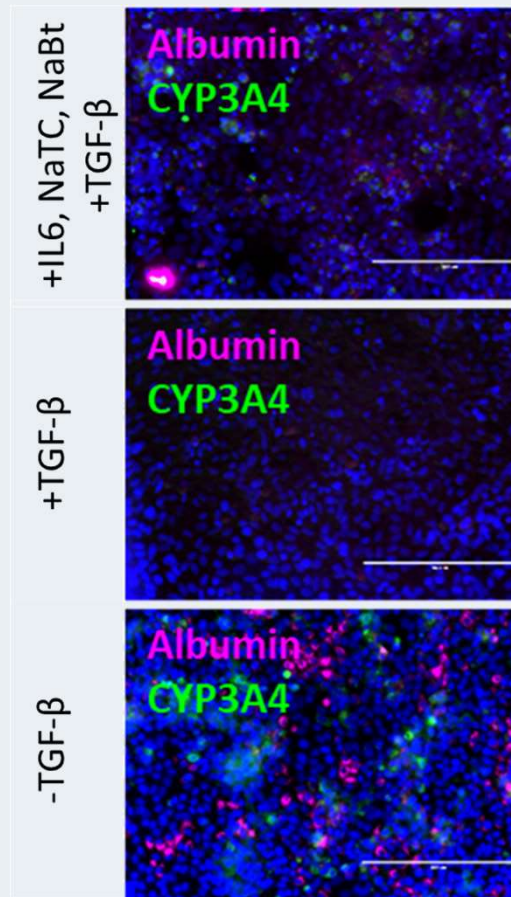
Dianat *et al*, (2014) *Hepatology*



Cholangiocyte differentiation

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Dianat *et al*, (2014) *Hepatology*

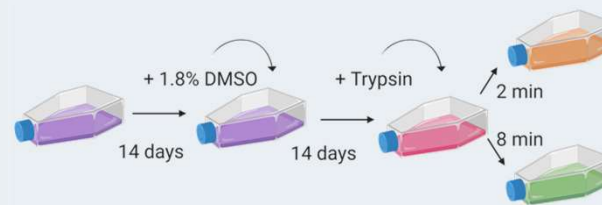


+/- TGF-β → prevention of hepatic differentiation

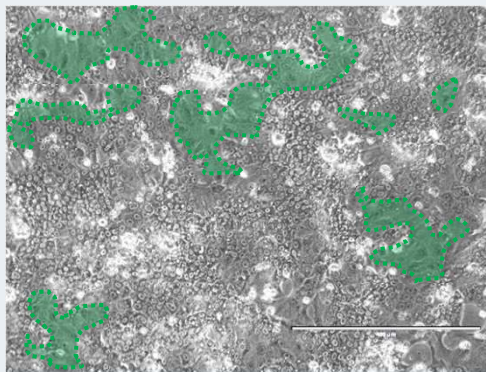
Currently optimising

HepaRG cells are heterogeneous

2. Can HepaRG cells be differentiated towards a cholangiocyte-like cell specific population?

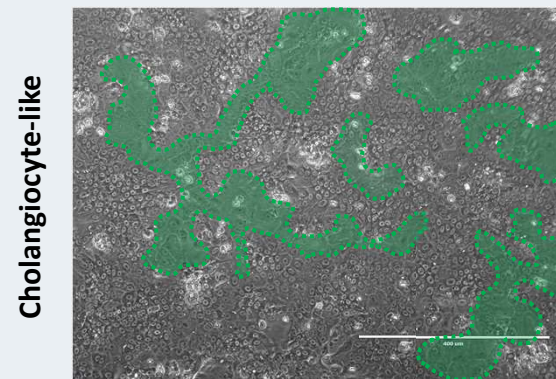


D28



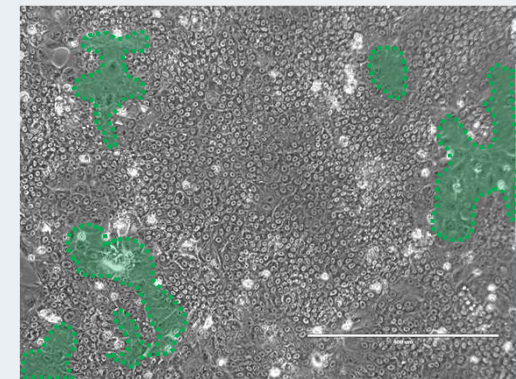
14 days culture medium
14 days + 1.8% DMSO

D56



Dedifferentiate
28 days + 1.8% DMSO

D56



Dedifferentiate
28 days + 1.8% DMSO

We can select HepaRG clones predisposed towards cholangiocyte-like cells shown by morphology

Differentiation

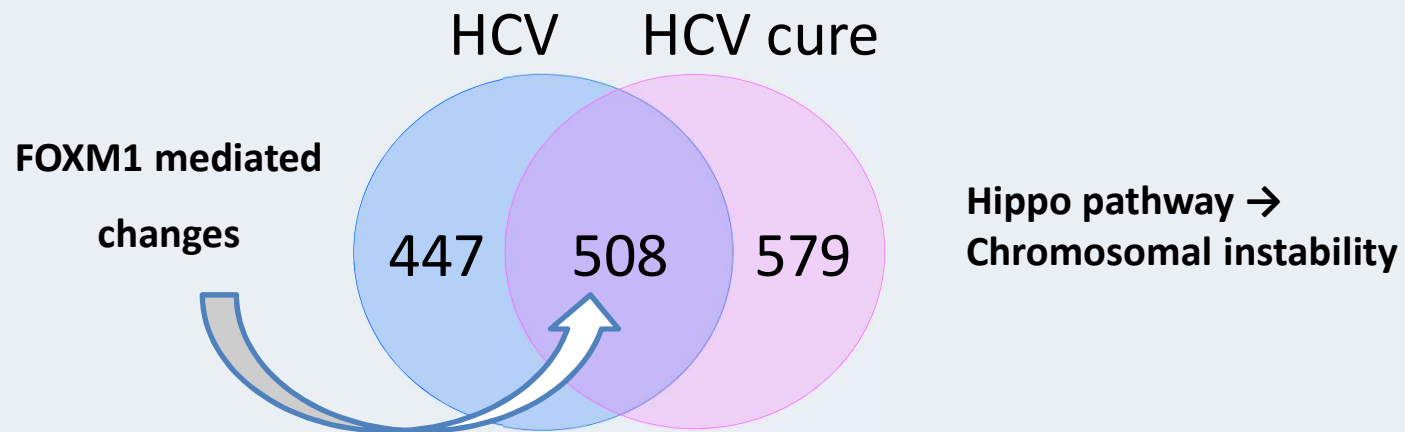
- PCR → cholangiocyte differentiation
- Trypsin isolation creates ↑cholangiocyte-like cells → isolate further using flow cytometry
- Move to iPSCs → established methods differentiation and literature on HCV infection

Model → determine the role of HCV in HPC differentiation and a iCCA pre-cancerous state.

Which pathways could be involved?



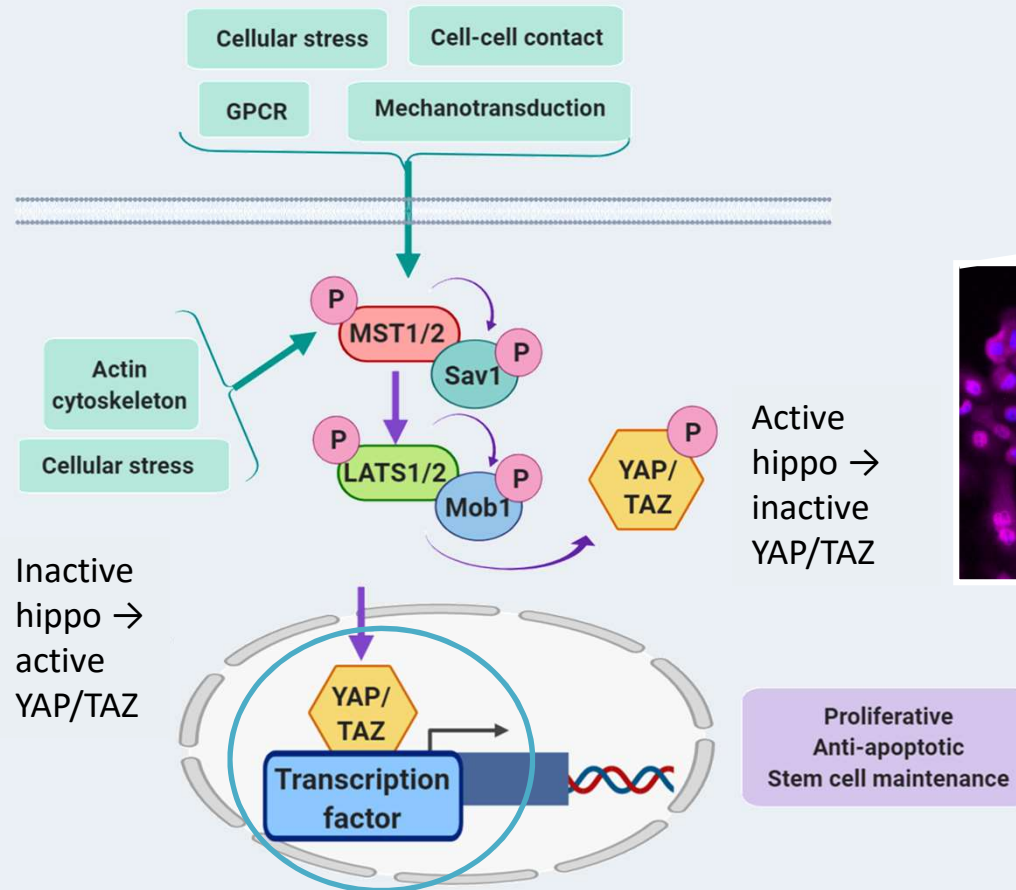
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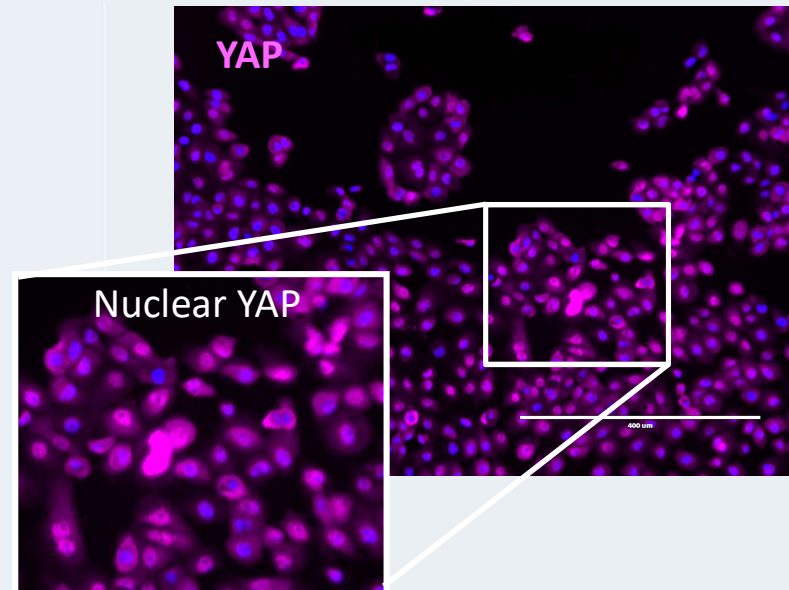
The hippo pathway



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HuCCT1: metastatic CCA cell line

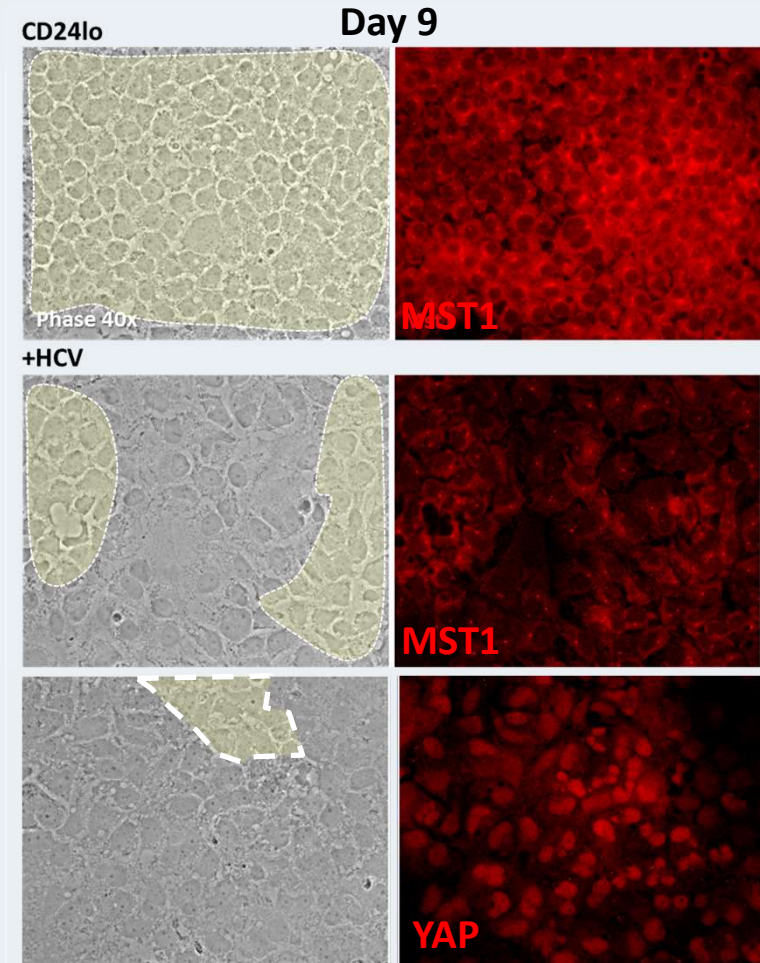
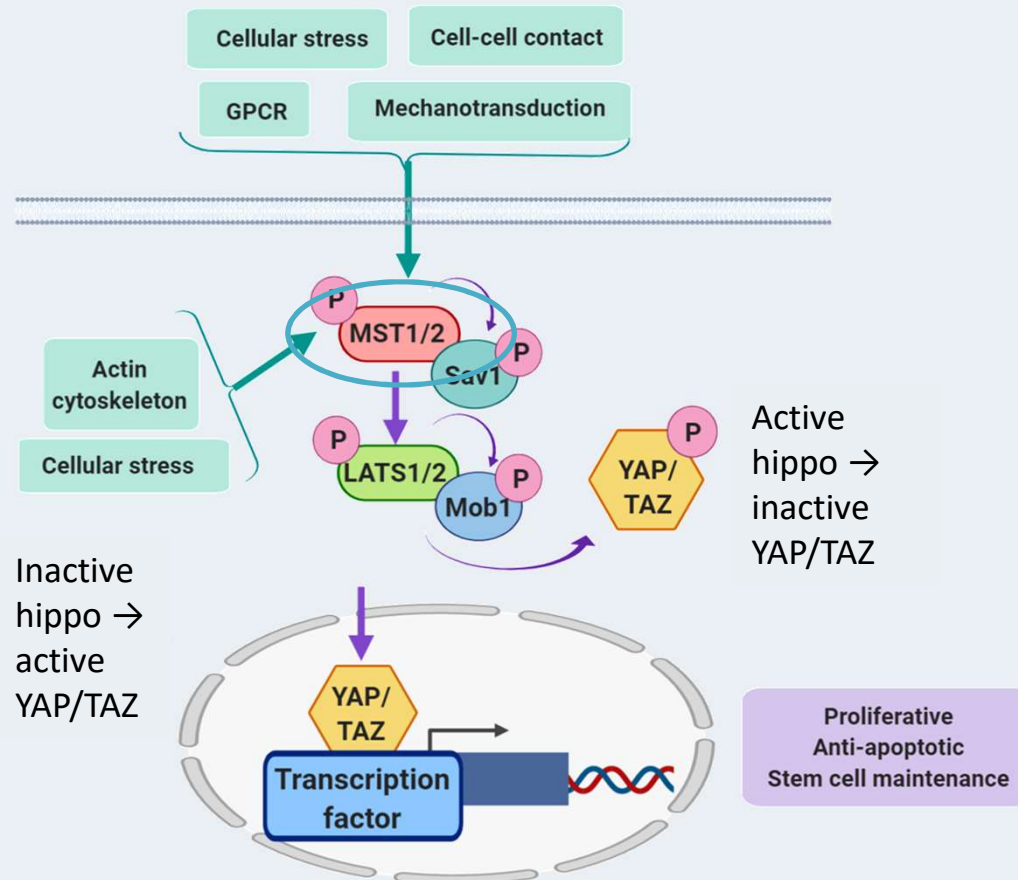


Inactive in various cancer types
→ known driver of iCCA

The hippo pathway



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Huh7 CD24lo hepatocyte
differentiation cell model

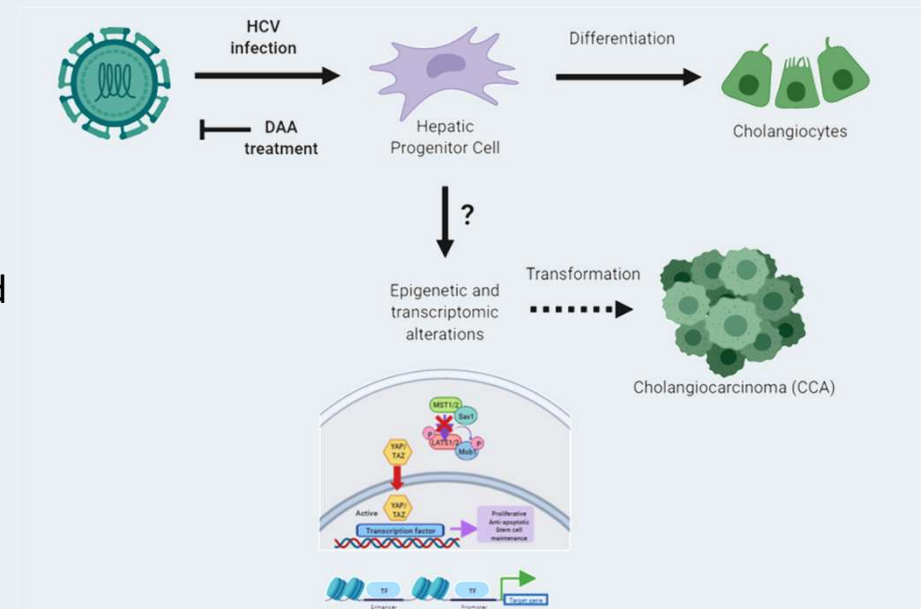


Summary



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- ↑iCCA in westernized world → related to HCV infection?
- DAAs do not eliminate the risk of developing PLCs → HCV epigenetic 'hit and run'
- HPCs can be infected with HCV → origin of HCV driven iCCA?
- Cholangiocyte-differentiation model using HepaRG cells → currently optimising
- Use this model to investigate the pathways linked to HCV epigenetic changes
- Hippo pathway involved?



Acknowledgements

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