HBV DNA integration: Consequences for Viral Gene Expression and Cellular Gene Dysregulation

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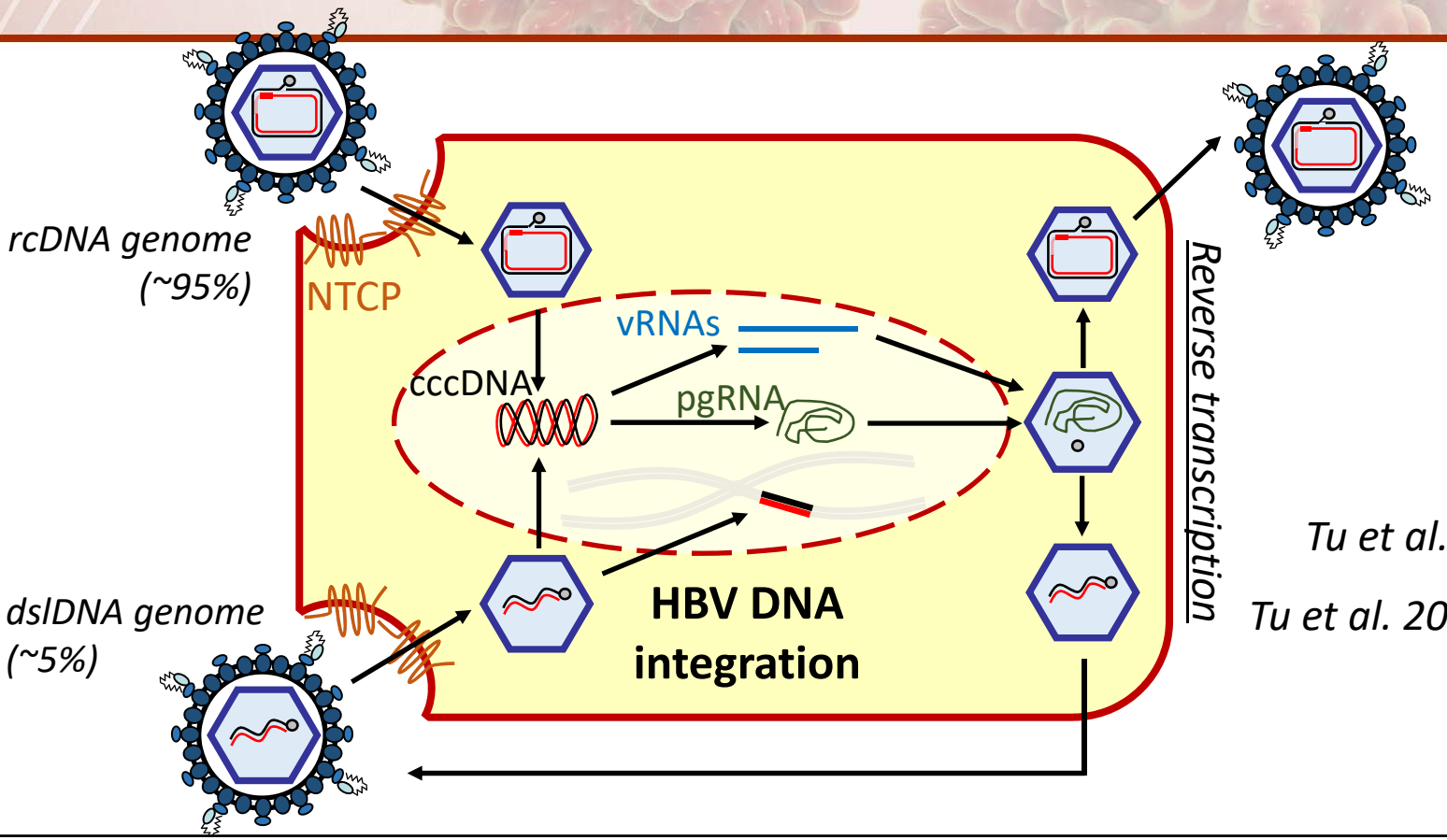
HBV replication



Tu et al. 2018, J Virol

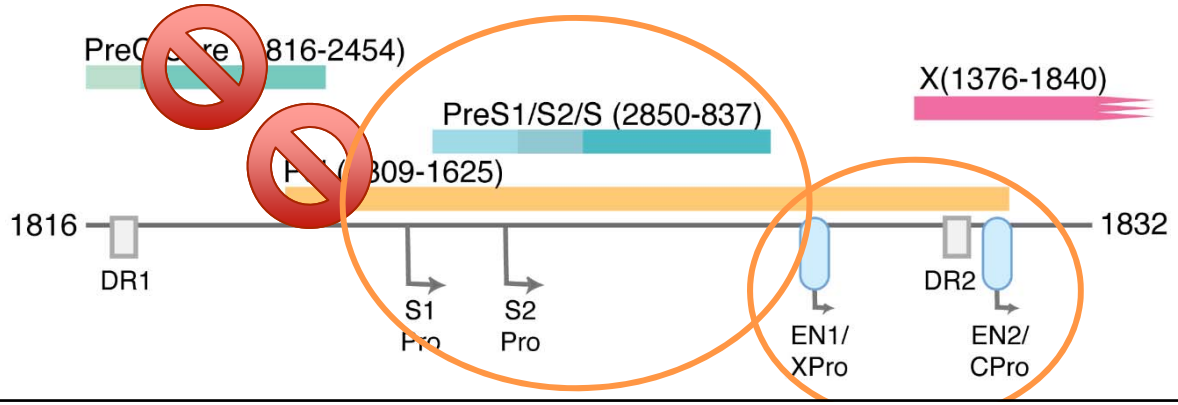
Tu et al. 2021, JHEP Rep

HBV DNA integration

- Random site in host genome at low rates (1 per 10^4 cells)
- Integrated HBV DNA is linked with HCC (>60% tumours)
  - Can drive expression of downstream genes –> *cis*-activation
  - May code for mutated proteins -> *trans*-activation

Tu et al. 2015, Liver Int.; Tu et al. 2017, Biol. Chem.

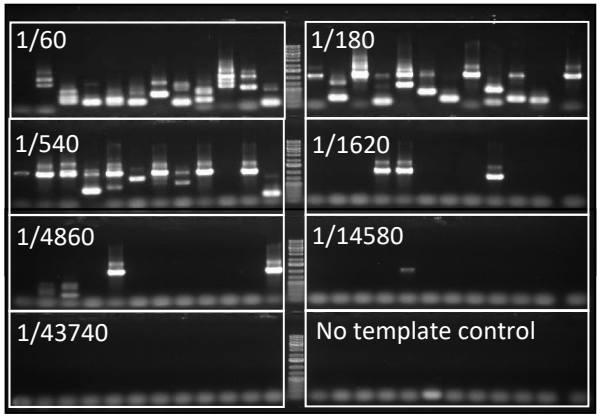
Integrated dslDNA (replication-deficient)



Tu et al. 2018, Viruses

Inverse nested PCR to detect HBV integration

Extract & sequence bands to confirm virus-cell junction



Gel electrophoresis

Yang and *Summers* 1999, JVI
Mason et al. *2010, JVI*
Tu et al. *2015, JVH*
Tu and Urban 2018 JoVE
Tu et al. *2018, JVI*
Tu and Urban 2018 JoVE

Integration frequency determined by end-point titration

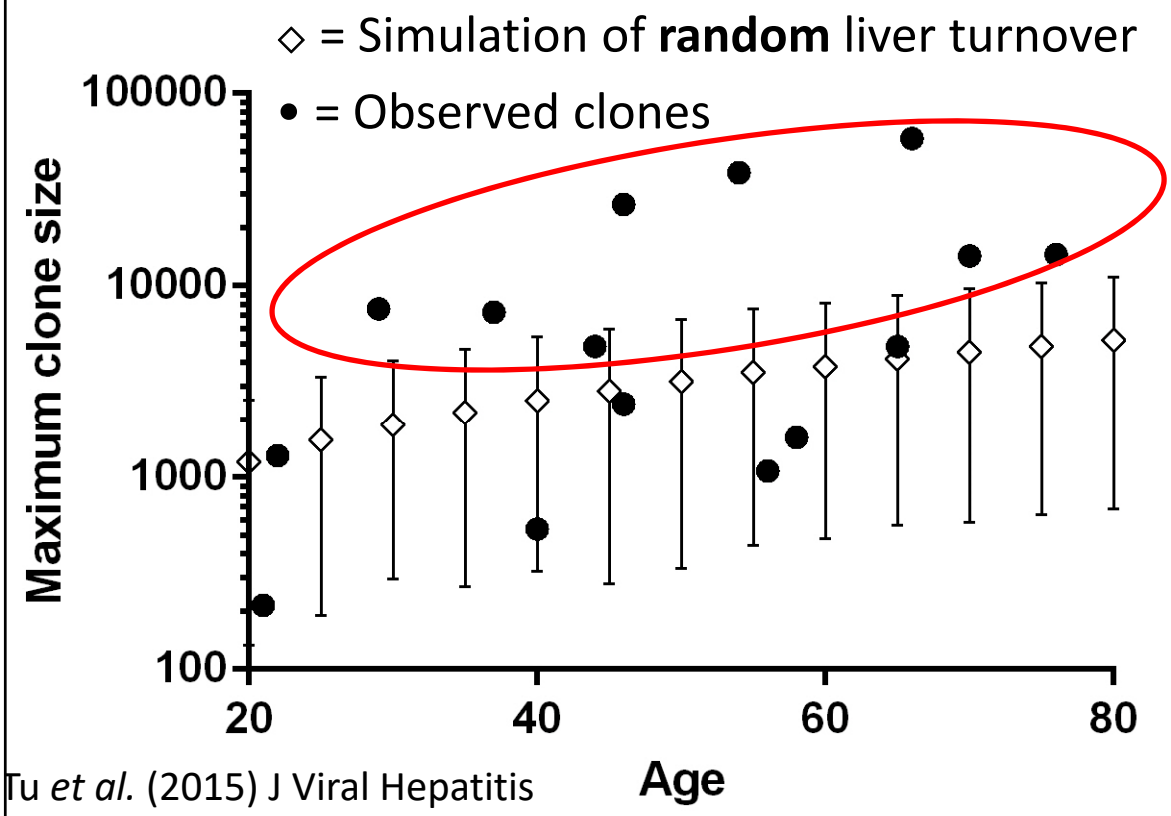
Serially dilute DNA prior to nested PCR

Quantitative

Unique junctions = clonal fingerprint

Repeated integration sites = clonal expansion

Cells with HBV integrations clonally expand



Tu *et al.* (2015) J Viral Hepatitis

Clones quantified in non-tumour liver of 18 HBV patients with invPCR

Clones with selection advantage detected

Likely risk factor for cancer

Hypotheses

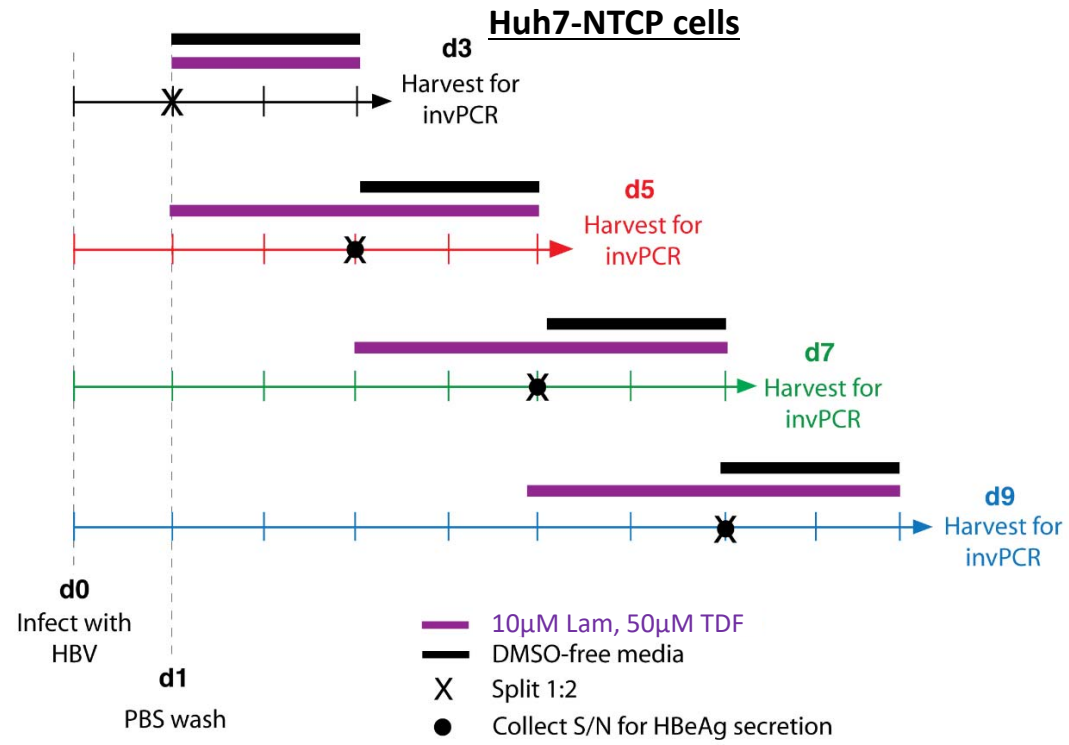
Selection advantage of hepatocytes is driven by HBV DNA integrations via:

1) *cis*-mediated (site-dependent) mechanisms

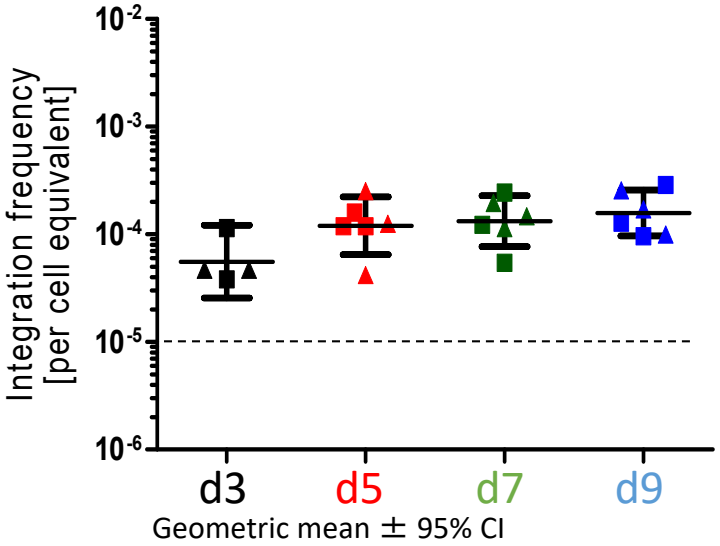
2) *trans*-mediated (site-independent) mechanisms

3) Associative (not causative) mechanisms

Null hypothesis: integrations before expansion



Integrations occur <3dpi and do not increase over time



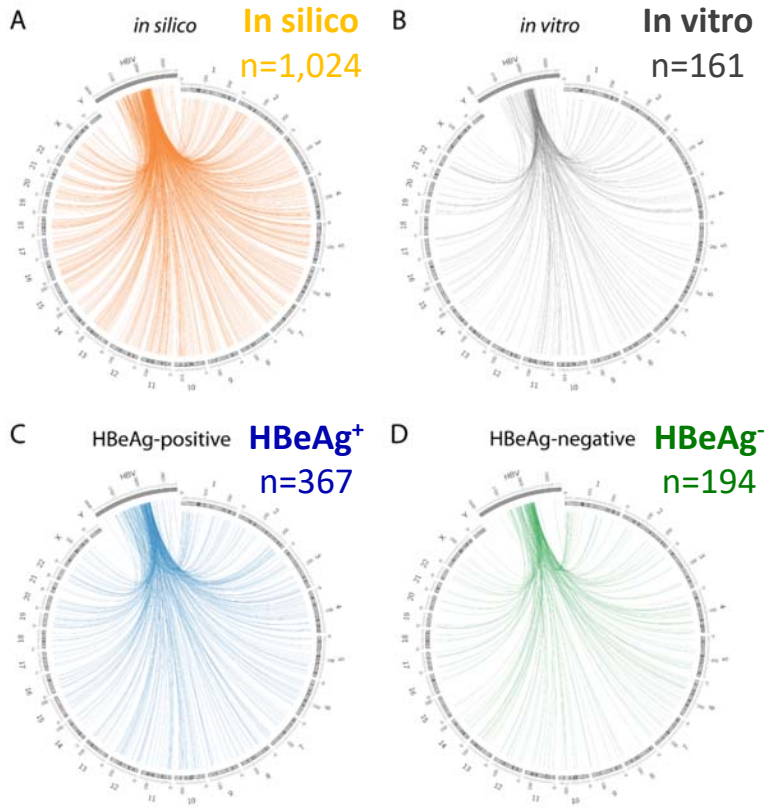
Tu et al. *2018, J Virol*

Expansion not likely due to *cis*-mediated mechanisms

No enrichment in:

- Expressed vs. non-expressed genes
- Transcriptional start sites
- Functional pathways

Where integrations occur does not play a role!



Magdalena Budzinska
PhD

Budzinska, ... Tu, 2018, Emer. Micro. & Infect.
Budzinska, ... Tu, 2018, Genes

Expansion not likely due to *trans*-mediated mechanisms

From 24 tumour and 63 non-tumour samples from HBV patients:

- Detected 43 integrations in total
- 6 and 7 highly clonal integrations in non-tumour and tumour
- Specific Sanger sequencing of integrated HBV DNA show no mutational differences between tumour and non-tumour

Monica Pinkerton
Honours student

What integrates does not play a role!

Tu et al., in preparation

HBV DNA integration frequency and HCC risk

- 33 anti-HBc+ and HBe^- patients (~20% on NA)
- Analysed non-tumour liver tissue

Group 1A = $HBsAg^+$ w/o HCC (n=3)

Group 2A = $HBsAg^+$ w/ HCC (n=15)

Group 1B = $HBsAg^-$ w/o HCC (n=6)

Group 2B = $HBsAg^-$ w/ HCC (n=9)

N.B. Liver from Groups 1A & 2B collected during resection of metastatic colorectal cancer.

Hung-Wen Tsai
Ih-Jen Su
Chiao-Fang Teng

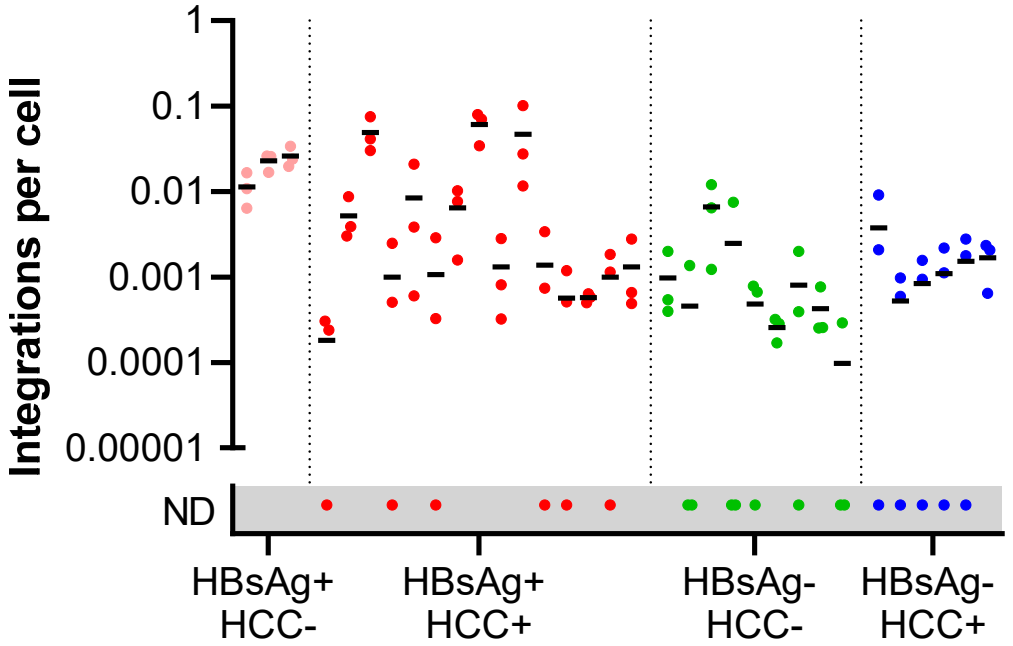
Tu et al., in preparation

Integration frequency **not** linked to HCC

Integration frequency in non-tumour liver not associated with:

- HBsAg-loss
- HCC occurrence

No clear associative link



Tu et al., in preparation

Are HBV integrations associated with phenotypical cellular changes?

TECHNISCHE UNIVERSITÄT MÜNCHEN

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Sally Coulter
Research Officer

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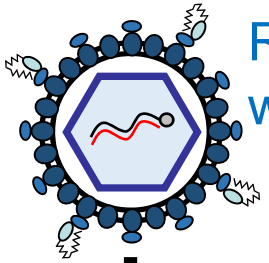
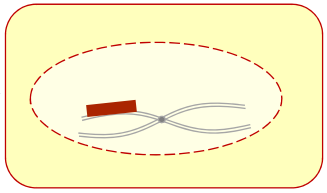
Q: Are cells with HBV integrations functionally different?

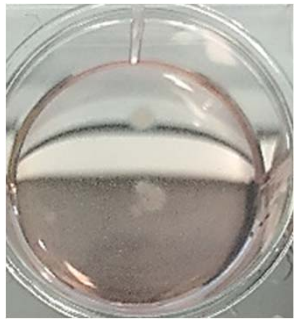


Reporter HBV with Zeo^{R}

HepG2-NTCP
Huh7-NTCP



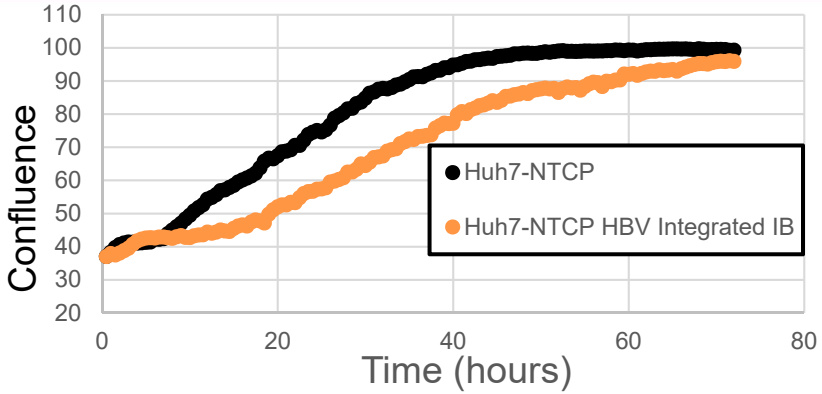
Zeocin selection



Characterise clones

Coulter et al., in preparation

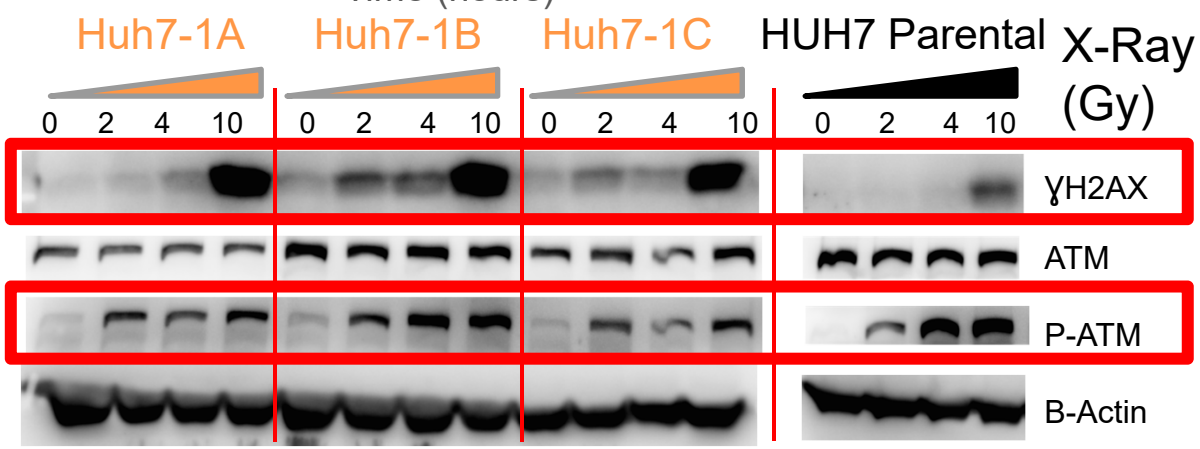
Cells with integrations grow slower, show more DNA damage, and less DNA repair



Slower growth by live-imaging

Higher ɣH2AX (DNA damage)

Poorer induction P-ATM (DNA repair)



Q: Does phenotype occur **before** or **after** integration?

Coulter et al., in preparation

Conclusions

Extensive clonal expansion occurs in cells with integration

HBV integration site, form, or frequency in non-tumour tissue does not appear to be associated with HCC

Still open question as to why HCC appear to contain more HBV DNA integrations than general hepatocyte population

- Are integrations more likely to occur in cells with oncogenic potential?

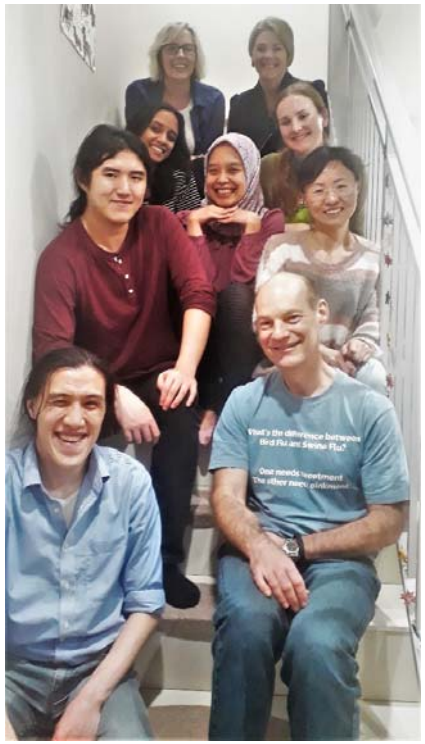
Acknowledgements

THE UNIVERSITY OF SYDNEY

STORR LIVER CENTRE

WESTMEAD HOSPITAL

Jacob George
Mark Douglas
Vikki Ho
Sally Coulter
Monica Pinkerton
Yuanyuan Liu
Rifqiyah Nur Umami
Vaishnavi Veeraraghavan
Henrik Zhang
Iram Aftab



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Florian Lempp
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UniversitätsKlinikum Heidelberg

Katrin Schöneweis
Lisa Walter

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Hung-Wen Tsai
Ih-Jen Su
Chiao-Fang Teng

TECHNISCHE UNIVERSITÄT MÜNCHEN

Ulrike Protzer
Jochen Wettengel

Funding:

Australian Government
National Health and Medical Research Council

DZIF
German Center for Infection Research

ACH2
Australian Centre for HIV and Hepatitis Virology Research