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# An integrative experimental and computational twin modeling approach to understand clonal dynamics in the normal liver

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How homeostasis is maintained in the liver remains the subject of debate, often driven by observations of replenishment after liver injury. Some authors report self-replication of hepatocytes,<sup>1</sup> others a contribution of biliary cells, especially in late-stage disease.<sup>2</sup> However, the homeostatic situation may differ from the organ response after liver injury.

Early studies utilized<sup>3</sup> H thymidine to track hepatocytes in rat livers over time,<sup>3</sup> showing hepatocyte migration along the portal tract (PT)-central vein (CV) axis, a phenomenon called ‘the streaming liver’.<sup>4</sup> Subsequent studies proposed neohepatocyte generation from all three zones in the hepatic lobule of the mouse liver: periportal,<sup>5</sup> midzonal<sup>6</sup> and pericentral.<sup>7</sup> Further still, there is evidence that homeostatic turnover results from zone-agnostic, broadly distributed hepatocytes.<sup>8,9</sup> Besides rodent models, there is evidence supporting the existence of a periportal progenitor niche in the human liver; however, this is in the context of end-stage liver disease when the majority of hepatocytes have been lost, or in the context of organoids generated from adult bile duct-derived bipotent progenitor cells.

In this issue, Passman *et al.*<sup>10</sup> have elegantly addressed the question of homeostatic liver cell renewal in the human liver using a systems medicine approach, integrating experiments and computational modeling in a simplified digital twin.

They applied a rich combination of experimental techniques to “healthy” liver tissue samples, mainly collected from patients undergoing surgery for colorectal cancer liver metastases. Their aim was to study to what extent a stem cell niche may exist, and to determine if physiological hepatocyte turnover in liver tissue homeostasis emanates from the PT, midzonal hepatocytes, or around the CV. (1) Elegantly, mutations permitting the histochemical identification of clonal patches of the mitochondrial enzyme cytochrome c oxidase (CCO) were used to trace the lineage of individual cell clones.<sup>11</sup> Thus, in 17 human livers analysed, an average of 76.9% of patches were located closer to PTs than CVs,

21.5% were closer to CVs, and 1.6% were sufficiently large to associate with both structures, supporting the hypothesis of the presence of a periportal stem/progenitor niche. (2) In-line with such a scenario, SOX9+/KRT19-hepatocytes (KRT19 negativity excludes duct cells) were detected in significantly greater abundance within 50 μm of PTs. (3) With CpG methylation sequencing, the mitotic age and therewith the ancestral relationship between the cells of a patch was determined.<sup>12</sup> The larger the methylation diversity, the more distant the ancestral relationship. This method showed increased methylation variability with patch-abuttal size at the PT but not at the CV, indicating that cells at the PT originate from a single stem cell located at the PT. However, the resolution of the methodology turned out to be insufficient to determine a possible increase of the epigenetic distance with anatomical distance from the stem cell niche. (4) A strong pillar of the analysis emerged from mitochondrial DNA (mtDNA) next-generation sequencing (NGS), used as a marker of cell lineage history within identified patches. MtDNA accumulates mutations, even in the absence of cell division. Hence, studying their variance in patches known to share the same origin (e.g. one CCO-deficient clone) along and perpendicular to the PT-CV axis allows one to infer information on the lineage history of the clone and its spatial temporal dynamics. The authors distinguish public variants as those present in all samples along the portal central axis of a CCO-deficient patch from private variants, defined as those not shared between neighbouring pairs of cuts, *i.e.* observed only in a subset of samples along the portal-central axis (Fig. 1A). Given the analysed cells share the same ancestor, the mtDNA in the clone should display increasing variants with increasing distance of the cell from the PT in physical space, if the clone emerged from the PT by continuous streaming. In case the streaming was fast, public variants characterized by a high degree of similarity between cells adjacent in space would

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be expected. Instead, the authors observed a large percentage of unique private variants, supporting the conclusion that patches grow in a discontinuous, punctuated fashion with intermittent quiescent phases in a way that the dynamics could permit cells in neighboring patches to develop sufficient independent mitochondrial variants.

In summary, CCO-deficient patch location, SOX9+ hepatocyte proximity to the PT, together with methylation diversity strongly support the existence of a periportal progenitor niche as the origin of clonal hepatocyte expansion. Further, the type of mtDNA variants determined by NGS strongly supports the hypothesis of punctuated cell movement with intermediate quiescence.

However, the plausibility of a set of mechanisms alone is not necessarily sufficient to explain the data qualitatively and quantitatively, as has been demonstrated, for example, in the context of ammonia detoxification, where modeling uncovered a mismatch between consensus mechanisms and data.<sup>16</sup> A corrected model guided researchers towards a possible therapeutic option for hyperammonemia.<sup>17</sup>

Along these lines, Passman *et al.*<sup>10</sup> complemented their sophisticated experimental analysis using computer experiments in a highly simplified digital twin of a tissue section around a portal triad (Fig. 1A), simulating the formation of cell clones wherein cell proliferation can occur either at the PT only, or homogeneously and isotopically distributed over the liver lobule, or via a combination of both. The spatial distribution of proliferation events determines the displacement dynamics of the cells. This, in combination with simulated mtDNA mutations occurring in each cell, determines the spatial distribution of mtDNA at each time point.

Passman *et al.*<sup>10</sup> applied an agent-based model simulation approach,<sup>18</sup> in which each agent represents a single cell. They distinguished PT cells and hepatocytes outside the PT.

In model set up 1, the position of each hepatocyte is explicitly represented in a circular simulation domain, while PT cells occupy a single position in space serving as a reservoir of ( $N_{PT}$ ) PT cells in the center of the domain corresponding to a two-dimensional (2D) cut section of a tissue with a portal triad in the middle of the section (Fig. 1A). A parameter  $\beta$ , defined as the division rate per cell at the PT divided by the division rate per cell not at the PT, controls the spatial distribution of divisions.

In the first simulation phase, 50% of the hepatocytes inside the simulation domain were depleted. In a second phase, the domain was replenished by cell division for chosen parameters  $\beta$  and  $N_{PT}$ . Dividing cells are able to push their neighboring cells towards the nearest vacancy left by a dead (=depleted) cell.

This depletion and replenishment cycle is repeated over several rounds. During the replenishing phase the cells accumulate mutations in the mtDNA through two processes. First, as a result of normal mitochondrial turnover throughout the cell cycle and, second, through cell divisions. Accordingly, alongside inheriting all mutations carried by the parent cell, daughter cells may also acquire new mtDNA mutations immediately after cell division.

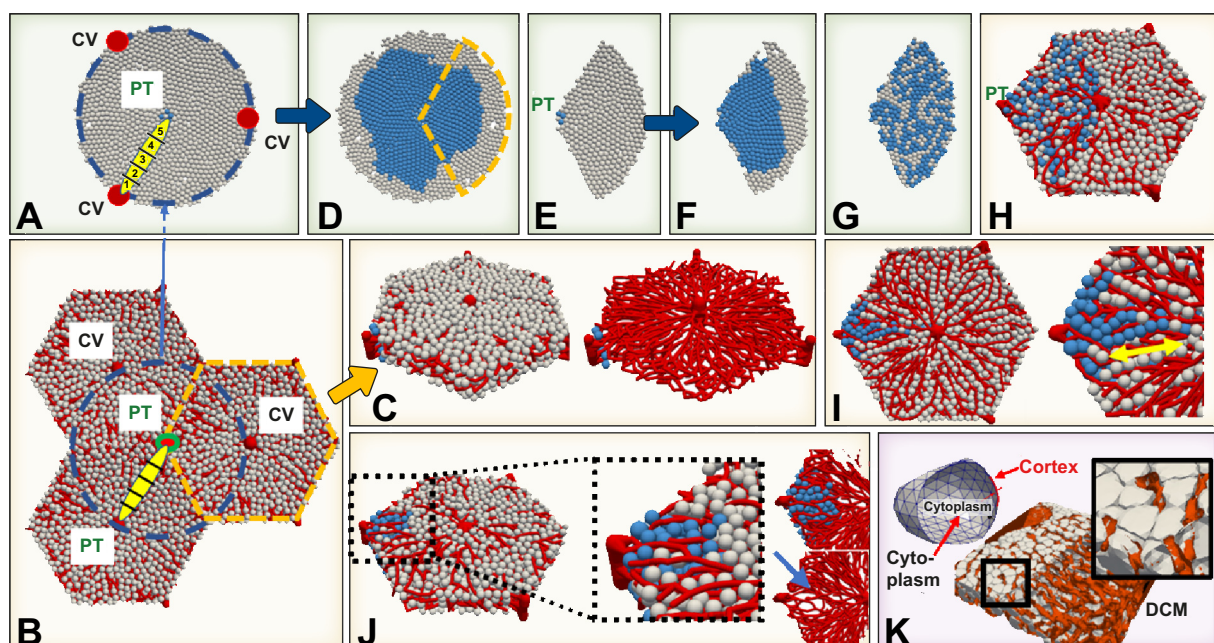
Simulations combined with Bayesian inference were used to estimate the best parameter combination ( $\beta$ ,  $N_{PT}$ ) for each patient individually via comparisons with their experimental data. This allowed the authors to estimate the ratio of cells dividing at

the PT vs. not at the PT, not directly accessible experimentally: Depending on the patient and the stained patch:  $\sim 1.2 < \beta < \sim 60$  and  $\sim 70 < N_{PT} < \sim 160$ . The finding of  $\beta > 1$  supports the experiment-based conclusion that a PT cell divided with higher chance than a non-PT cell. Based on typical values of liver lobule size (1-1.5 mm), hepatocyte diameter (20-30  $\mu\text{m}$ ), the association of a circular region as in Fig. 1A to one PT (so that  $N_{nonPT} \approx 4,000$ ), and typical values for  $\beta$  and  $N_{PT}$  of 20 and 100, respectively, the chance of observing a cell division at the PT vs. not at the PT in a snapshot would be about the same.

In model setup 2, representing only the (one dimensional) PT-CV axis (Fig. 1A) to limit computation time, the clonal expansion dynamics were simulated. The question was whether a punctuated expansion with intermittent quiescent phases or conveyor-like streaming dynamics would better reproduce the experimental NGS data, in which mtDNA had been analyzed in 5 cuts along the PT-CV axis (Fig. 1A). The high frequency of private vs. public variants found in the simulations support the conclusion of punctuated clonal expansion with long periods of quiescence observed in the experiments, whereby for the studied patient group, a slow expansion with 20 years quiescence fitted the data best.

While models may help with data interpretation,<sup>10,19</sup> to infer parameters not directly accessible by experiments or data acquisition,<sup>10</sup> to uncover inconsistencies or a lack of elements in sets of mechanisms,<sup>16</sup> and to simulate their consequences if technically well realized,<sup>17</sup> they cannot prove the correctness of a mechanism.

The particular challenge in the study by Passman *et al.*<sup>10</sup> is the time scales (decades), which prompted the authors to use highly simplified models. However, high simplification bears the risk of artifacts, as the authors correctly noted. In their study,<sup>10</sup> several simplifications could modify the spatial distribution of mutations through altered clone shapes: *e.g.*, (1) the clonal simulations were performed on a 2D rigid square lattice permitting horizontal, vertical and diagonal cell displacements only, thereby risking lattice artifacts;<sup>20</sup> (2) the cell cycle time duration distribution was Poissonian (decreasing exponential), strongly advantaging the formation of clones from cells with shorter-than-average cycle times, while true cell cycle times usually peak sharply around an average;<sup>20</sup> (3) the clonal growth and cell movement dynamics were mimicked by a rule-based stochastic process, which risks overlooking physical effects (Fig. 1J), moreover, how many cells would fit to a PT in a 2D section (ideally perpendicular to the PT orientation given the model set up) is not so clear; (4) sinusoids were neglected, which may modify the clonal shapes and hence the mutation pattern (Fig. 1F vs. 1I). To illustrate possible effects of geometry and lobule microarchitecture on clonal expansion, we ran clonal replenishment simulations after a single 50% depletion with a CBModel in a 2D circle geometry (Fig. 1A,D-G) and in a 3D liver lobule microarchitecture (Fig. 1B,C,H-J). In a CBModel, each cell is mimicked by a sphere-like elastic adhesive object that can take any position in space and moves as a result of all physical forces acting on it and its own micro-motility.<sup>13,14</sup> For division of only non-PT-cells ( $\beta = 0$ ), the clonal shapes in the 2D circle and 3D lobule are equal (Fig. 1G, H). For cell division at the PT only ( $\beta \rightarrow \infty$ ), the clone preferentially expands along the sinusoids (Fig. 1I,J). The clone shape markedly differs from that in a 2D simulation with no sinusoid scaffold (Fig. 1D,F). If the



**Fig. 1. Modelling clonal replenishment dynamics in tissue homeostasis in the healthy liver.** (A) Sketch of the model set ups in Passman *et al.*,<sup>10</sup> where the cells are instead represented by spherical objects in free space. In model set up 1 of ref.,<sup>10</sup> the PT is localized in the center of a 2D-circular simulation domain, which may be associated to three adjacent liver lobules connected by one portal tract/triad and CVs localized at about equal distance from the PT (B). (C) Side view for rightmost model liver lobule in (B), constructed from confocal microscopy (mouse data,<sup>15</sup>). The light-yellow domain in (A, B) corresponds to the one-dimensional model set up 2, used by the authors to test the streaming liver vs. punctuated expansion with quiescent phases – dynamics. Five adjacent tissue patches were examined for private vs. public mtDNA variants. (D–J) Simulations with a CBModel, which models cells as spherical objects<sup>14</sup> and text), to illustrate the potential role of scaffold structures due to sinusoids (missing in<sup>10</sup>) and of biomechanics (highly simplified in<sup>10</sup>) on clonal expansion. (D) The simulation result after one simulated depletion (50%) and subsequent replenishment cycle starting from (A) by cell division only at the PT (blue patch=clone, mtDNA mutations were not simulated). Starting from (E), the patch in (F) looks as in (D) in a 1/3-circle that corresponds to one liver lobule (dark yellow in (B, D)). (G) Clone shape after one depletion/replenishment cycle starting from (E) if cell division occurs isotropically and homogeneously distributed only by non-PT cells in 2D, and (H) in the 3D lobule microarchitecture of (B, C). (I) Clone shape if only PT cells divide in the 3D lobule-microarchitecture (B, C), where the forming clone has a tendency to orient along the sinusoids, here along the PT–CV axis. (J) If the spatial reorganization process is too slow to relax the physical pressure building up, the sinusoidal network may be locally deformed (blue arrow). (K) A computational cell model explicitly resolving cell shape (DCM) permits cells to adapt their shapes to the sinusoidal structure<sup>15</sup>. CV, central vein; mtDNA, mitochondrial DNA; PT, portal tract; 2D, two-dimensional.

spatial reorganization process is too slow to relax the physical pressure building up during clonal expansion, the sinusoidal network may be modified locally (Fig. 1J). This illustrates why considering biomechanical parameters can be important. Pressure build-ups as in Fig. 1J may also be a consequence of the massive 50% depletion and replenishment cycle, and could disappear if clonal expansion occurs cell-by-cell.

Nevertheless, a large influence of the model simplifications made in<sup>10</sup> on the clonal dynamics may not be expected: the mixed clonal dynamics of PT/non-PT cells weaken the sinusoidal effects, and many depletion/replenishment cycles, as well as the finding of a punctuated expansion with intermittent quiescent phases blur out sharp clonal borders. In principle, one could think of simulations performed directly out of patient histology in 3D using models that explicitly resolve cell shape and reflect cell mechanics (Fig. 1K). As such, models are very

costly to implement and a community effort to generate digital liver twin software is warranted. Simulations could then test mechanisms in silico that resemble what would be expected to be seen in intravital microscopy, similarly to tools used in bio-informatics. In the context of liver tissue replenishment, such simulations would permit the study of entire regeneration scenarios based on an implemented mechanistic hypothesis both after acute<sup>13,14</sup> and in chronic damage. They would include parenchymal and non-parenchymal cells, inter- and intracellular signals, and the production of biomarkers as histopathological readouts. Alongside high-resolution imaging modalities, such as temporal diffusion spectroscopy which could be used both as a direct source of information and to parameterize the digital twin (models), such an endeavor could be a step towards a tool to inform physicians about processes likely ongoing at the histological level in a certain patient.

#### Affiliations

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## Conflict of interest

The authors declare no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

DD wrote the manuscript and conceptualized the simulations, JZ performed the computer simulations.

## Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2023.05.016>.

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*Author names in bold designate shared co-first authorship*

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