



Spatial metabolism uncouples clonal fitness from liver cancer initiation

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INTRODUCTION

Somatic mutations in histologically non-malignant tissues challenge the assumption that the largest positively selected clone poses the greatest cancer risk. In chronic liver disease, repeated injury and regeneration generate numerous mutant hepatocyte populations, yet only a subset progresses to hepatocellular carcinoma (HCC). Guo *et al.* showed that spatial metabolic context shapes the fate of hepatocytes carrying oncogenic alterations^[1]. Beyond the reported link between zonation, glutathione S-transferases and ferroptosis^[2], these findings distinguish clonal persistence from malignant transformation.

Here, ecological fitness denotes a mutant clone's capacity to persist or expand relative to neighboring hepatocytes within a given tissue environment. Transformation competence denotes the capacity of mutant cells to undergo malignant conversion and initiate a tumor. Spatial metabolic context can decouple these properties by supporting clonal maintenance differently from the stress resistance and proliferative programs required for transformation.

METABOLIC ZONATION SEPARATES CLONAL PERSISTENCE FROM TRANSFORMATION COMPETENCE

Guo *et al.* used *Gls2*-CreERT2 and *Cyp1a2*-CreERT2 mice to induce mosaic *Ctnnb1* activation and *Arid2* loss^[1]. These systems target periportal zone 1 and a pericentral-biased compartment extending into zone 2, respectively. Ectopic glutamine synthetase (GLUL) expression, tdTomato labeling, β -catenin localization, spatial transcriptomics and single-nucleus genotyping were combined to follow mutant cells *in situ*. Zone 1 mutant hepatocytes persisted and showed greater proliferative activity, whereas most mutant cells in the *Cyp1a2*-labeled compartment progressively disappeared. Nevertheless, visible tumors developed in 90% of *Cyp1a2*-targeted mice, compared with 59% of *Gls2*-targeted mice; malignant lesions were also more frequent



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in the former group^[1]. A pericentral bias was observed across an *Arid2* allelic series, with *Ctnnb1* activation alone, and in diethylnitrosamine and oncogenic NRAS/*Tp53*-knockdown models^[1].

These findings show why ecological fitness and transformation competence should be assessed separately. In the models studied by Guo *et al.*, zone 1 favored clonal persistence, whereas the broader *Cyp1a2*-labeled compartment had greater tumor-initiating potential^[1]. Human studies have identified positively selected liver clones enriched for mutations in metabolic and stress-response genes^[3-5]. A large clone may therefore reflect adaptation to tissue injury without having the greatest malignant potential. Conversely, a small clone may warrant attention if its local metabolic state facilitates transformation. Neither clone size nor variant allele fraction alone captures this distinction.

GSTM2 AND PARTICULARLY GSTM3 CONTRIBUTE TO A REDOX GATE FOR TUMOR INITIATION

Glutathione S-transferase mu 2 (GSTM2) and mu 3 (GSTM3), which are enriched pericentrally, provide a mechanistic link between zonation and tumor initiation. Genetic disruption of *Gstm2* or *Gstm3* reduced early tumor formation; transient knockdown of *Gstm3* using small interfering RNA (siRNA) also reduced early tumor burden^[1]. In a CTNNB1/MYC hydrodynamic transfection model, zone 1-restricted *Gstm3* overexpression increased the proportion of zone 1-derived tumors from 22% to 61%^[1]. This intervention supports a causal role for GSTM3 in determining tumor origin within that model. WNT/ β -catenin-dependent regulation of hepatic glutathione transferase expression provides a physiological context for this effect^[6]. Oncogenically altered cells may exploit a detoxification program that normally protects pericentral hepatocytes.

Ferroptosis resistance provides one explanation for this effect. *Gstm3* loss reduced glutathione availability, increased lipid peroxidation and sensitized hepatocytes and HCC cells to glutathione peroxidase 4 (GPX4) inhibition^[1]. *Gstm3* overexpression had protective effects. In the rapid CTNNB1/MYC model, overexpression of ferroptosis suppressor protein 1 (FSP1) rescued tumor initiation after *Gstm3* deletion^[1]. FSP1 can suppress ferroptosis independently of glutathione^[7], supporting ferroptosis resistance as a mechanism linking GSTM3 activity to malignant conversion. This mechanism is unlikely to explain all GSTM-dependent effects. GSTM2 also regulates other stress responses, ethacrynic acid inhibits multiple glutathione transferase activities, and residual drug effects persisted after combined *Gstm2/Gstm3* loss^[1]. GSTM2 and GSTM3 therefore contribute to spatially biased HCC initiation partly through ferroptosis suppression, with stronger mechanistic evidence for GSTM3.

THE PERICENTRAL COMPARTMENT IS NOT A UNIFORM TUMOR-PERMISSIVE STATE

Raven *et al.* identified a complementary constraint on tumor initiation^[8]. In their CTNNB1/MYC models, an extreme perivenous differentiation state suppressed the proliferative translatome. The GLUL-positive subpopulation expressing leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) was refractory to CTNNB1/MYC-driven tumorigenesis. Early proliferative lesions instead showed reduced WNT activity and increased mitogen-activated protein kinase (MAPK) signaling. Their growth depended on insulin-like growth factor-binding protein 2 (IGFBP2), mechanistic target of rapamycin (mTOR) and cyclin D1 signaling^[8].

The studies differ in the populations, oncogenic combinations and biological endpoints emphasized. Guo *et al.* compared long-term persistence and tumor formation after mosaic *Ctnnb1* activation and *Arid2* loss in *Gls2*- and *Cyp1a2*-labeled compartments^[1]. Their CTNNB1/MYC hydrodynamic transfection experiments separately tested GSTM-dependent redox protection. Raven *et al.* combined sporadic oncogene activation

with several zonal Cre models to investigate differentiation, proliferation and tumorigenesis^[8]. Their *Lgr5*-targeted experiments specifically resolved the extreme perivenous subpopulation within zone 3. Thus, neither the targeted populations nor all cooperating alterations and endpoints were equivalent. Resistance of the narrow LGR5-positive compartment does not imply resistance of the broader *Cyp1a2*-labeled compartment.

Together, the studies suggest that distinct constraints operate within the pericentral region. GSTM-dependent redox protection may support survival during transformation, whereas an extreme WNT-high differentiation state may restrain proliferation. A permissive state might combine ferroptosis resistance with escape from perivenous differentiation. This remains a working interpretation, rather than a shared intermediate state directly demonstrated by the two studies. The central implication is that anatomical zone 3 contains functionally distinct hepatocyte populations.

HUMAN SPATIAL ATLASES REFRAME THE ZONAL ORIGIN OF HCC

Single-nucleus profiling and human spatial atlases provide complementary evidence for disease-associated changes in hepatocyte identity. *Carlessi et al.* identified a disease-associated hepatocyte state through single-nucleus RNA sequencing (snRNA-seq) in mouse models and subsequently validated related signatures in human chronic liver disease datasets, with evidence of prognostic relevance for HCC development^[9]. Multiplexed error-robust fluorescence in situ hybridization (MERFISH) and snRNA-seq identified hepatocyte populations that expand in fibrotic liver without clear zonal distributions^[10]. However, some periportal and pericentral populations retained their expected locations, indicating heterogeneous remodeling rather than uniform loss of zonation^[10]. A multi-omics study of 61 human livers identified fibrosis-associated programs and suggested profibrotic interactions between central-vein endothelial cells and hepatic stellate cells^[11]. Mouse experiments also showed that hepatic stellate cells regulate hepatocyte zonation through R-spondin 3 (RSPO3)-WNT signaling^[12]. Vascular and mesenchymal niches may therefore help maintain or remodel transformation-permissive metabolic states.

Two further human atlases define the limits of anatomical extrapolation. A live-donor atlas documented pronounced portal-to-central zonation and human-specific expression patterns^[13]. Spatial proteomics in a cohort of 18 individuals identified zonated expression in approximately half of the proteins analyzed in healthy hepatocytes^[14]. In the disease samples, regions adjacent to liver metastases showed desmoplastic disruption and loss of protein zonation, particularly among pericentral proteins^[14]. These findings illustrate the sensitivity of zonation to architectural disruption, but their applicability to cirrhosis requires direct validation. Together with evidence of heterogeneous hepatocyte remodeling in fibrotic liver^[10], they caution against assuming that normal lobular coordinates reliably identify metabolic states in diseased tissue. A GSTM-high, redox-protected state could persist, relocate or disappear as fibrosis progresses. Its existence and association with malignant risk require direct testing.

Spatial metabolomics is essential to test this model because transcript and protein abundance alone cannot establish the local metabolic environment. *Li et al.* combined spatial transcriptomics with mass spectrometry imaging and identified disease-associated phospholipid accumulation in human liver^[11]. Extending this approach to premalignant tissue could connect metabolite and lipid distributions with oncogenic mutations and transcriptional programs. Co-registered measurements should assess whether particular metabolic environments distinguish expanding but non-transforming clones from dysplastic lesions and early HCC.

Human validation should combine spatial metabolomics, transcriptomics and proteomics with laser-capture genotyping and somatic phylogenetic reconstruction. These analyses could test whether CTNNB1- or ARID2-mutant, GSTM-high clones disproportionately contribute to dysplastic nodules and early HCC.

Candidate measurements include glutathione redox balance, phospholipid composition, lipid-peroxidation products such as 4-hydroxynonenal, and GSTM2/3, GPX4 and FSP1 expression or activity. Spatial associations would need functional validation through metabolite or pathway perturbation to establish whether metabolic niches alter transformation competence. Sampling should include fibrosis stage and disease etiology, with attention to steatohepatitis, alcohol-associated liver disease and viral hepatitis.

LIMITATIONS AND TRANSLATIONAL PRIORITIES

The principal translational limitation is the disease context. The key mouse tumor-initiation experiments were not conducted against established chronic liver disease and do not reproduce the remodeled microenvironment of cirrhosis-associated human HCC. Their spatial hierarchy of susceptibility therefore cannot be assumed to persist during chronic inflammation, fibrosis and regeneration. Human spatial transcriptomics documents residual zonal identities alongside injury-associated hepatocyte states in fibrotic liver, whereas spatial proteomics demonstrates disrupted zonation in desmoplastic tissue adjacent to liver metastases^[10,14]. Validation must distinguish preserved anatomical zonation from redistributed metabolic states, rather than assign risk using normal lobular coordinates alone.

Several methodological boundaries also matter. The CreERT2 systems are zone-biased rather than strictly zone-specific; *Cyp1a2*-CreERT2 labeling extends into zone 2, and recombination efficiencies differ across reporter and target loci. GLUL is a useful readout of activated *Ctnnb1* but is not an independent lineage marker and is physiologically expressed around central veins. Orthogonal labeling, spatial profiling and genotyping strengthen the evidence, although the five-month single-nucleus genotyping comparison included only one mouse per zonal condition^[1]. The strongest *Gstm3* overexpression, *Fsp1* rescue and pharmacological experiments also used rapid CTNNB1/MYC hydrodynamic transfection^[1]. These findings require testing in models that incorporate chronic injury and fibrosis.

Ethacrynic acid remains a proof-of-concept intervention rather than an established chemopreventive agent. It lacks GSTM3 selectivity and may compromise physiological detoxification, glutathione homeostasis and renal electrolyte balance. Priorities include selective target validation in chronic fibrosis-associated models, spatial measurement of metabolic responses and long-term assessment of liver injury. Hepatocyte-directed or temporally restricted interventions could then test whether high-risk premalignant states can be targeted while preserving normal metabolic defenses.

CONCLUSION

Guo *et al.* show that mutation identity and clone size alone do not determine hepatocyte transformation in the models examined^[1]. Together with Raven *et al.*, their findings support spatially conditioned oncogenic penetrance as a framework linking genotype, differentiation, niche-regulated metabolism and stress resistance^[8]. Whether this framework explains HCC initiation in chronically diseased human liver remains unresolved. Integrating spatial metabolic measurements with clonal genotyping could test that possibility and clarify whether metabolic state adds predictive value beyond clone size.

DECLARATIONS

Authors' contributions

Conception and design: Wan K, Qian C, Liu L

Literature search and evidence verification: Wan K, Tang K, Tian Q, Wang L

Writing - original draft: Wan K

Writing - review and editing: Wan K, Tang K, Tian Q, Wang L, Qian C, Liu L

All authors approved the final version of the manuscript.

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Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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