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increasingly possible to test whether any within-site horse genetic ancestry variation can be explained by human social organization. This type of analysis would also be interesting to perform on known trait-associated alleles. That is, at what point(s) do we begin to observe evidence suggesting distinct roles for different types (i.e., phenotypes) of horses within the same spatiotemporal horizon?

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Spotlight

Beneficial Noncancerous Mutations in Liver Disease

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Chronic liver disease results in fibrosis and cancer. While injury is associated with mutational burden, a recent study (Zhu *et al.* *Cell* 2019;177:608–621) highlights that not all positively selected mutations in the liver are precancerous. Indeed, some may be beneficial to the ability of the liver to not only withstand injury , but also to regenerate.

Genetic mutations are a hallmark of cancer. Throughout biological systems, from cells to organisms, intrinsic and extrinsic factors drive population dynamics. A nonneutral drift in cell populations is seen in health and disease, including cancer. The general consensus is that mutations that are clonally selected within a tissue are inevitable stepping stones towards the development of cancer, and not beneficial to the host.

The role of the liver in detoxification and bile synthesis exposes it to frequent damage, which it copes with due to its great regenerative potential. However, chronic damage leads to the accumulation of mutations that can lead to hepatocellular carcinoma (HCC) [1,2]. If the liver is chronically damaged, the liver lobules become separated by dense fibrosis and nodules of hepatocytes form, which are believed to be clonal in origin [3]. During nodule formation, it is unclear which mutations drive clonal 'fitness'. Understanding whether mutations driving such clonal expansion always carry the risk of malignant transformation, or whether they may instead be beneficial to liver regeneration, has importance not only scientifically, but also medically in regenerative medicine, and early cancer detection and treatment.

In a recent landmark study, Zhu *et al*. dissected the malignant potential of mutations found in nondysplastic lesions of patients with chronic liver disease by using whole-exome sequencing, followed by targeted deep sequencing [4]. After carefully excluding malignant tissue and using a highly stringent methodology for calling mutations, they were able to identify recurrent mutations occurring in clonal outgrowths in multiple patients. Intriguingly, it appeared that some of these recurring mutations were not drivers of HCC because they have not been found in previous large-scale cancer genomic studies. Using an unbiased *in vivo* CRISPR screen in mice, the authors then confirmed their observational findings, showing that many of these recurrent mutations provided a selective survival advantage to hepatocytes and, thus, represent genuine driver mutations. This screen, targeting 147 mutated genes identified in the human samples, was performed using a genetic mouse model of metabolic deficiency. This allowed the selection of clones rescued from the metabolic deficiency and compared the regeneration fitness of these clones based on the additional targeted gene mutations. Finally, Zhu *et al*. tested the regenerative capability and resistance to both acute and chronic injury after hepatic knockdown of three of their top hits, *Arid1a*, *Kmt2d*, and *Pkd1*. They showed that each was capable of either improved regeneration, reduced susceptibility to damage, or both. In summary, the authors observed clear clonal dominance from these 'fitness-promoting mutations', which did not appear to be associated with cancer.

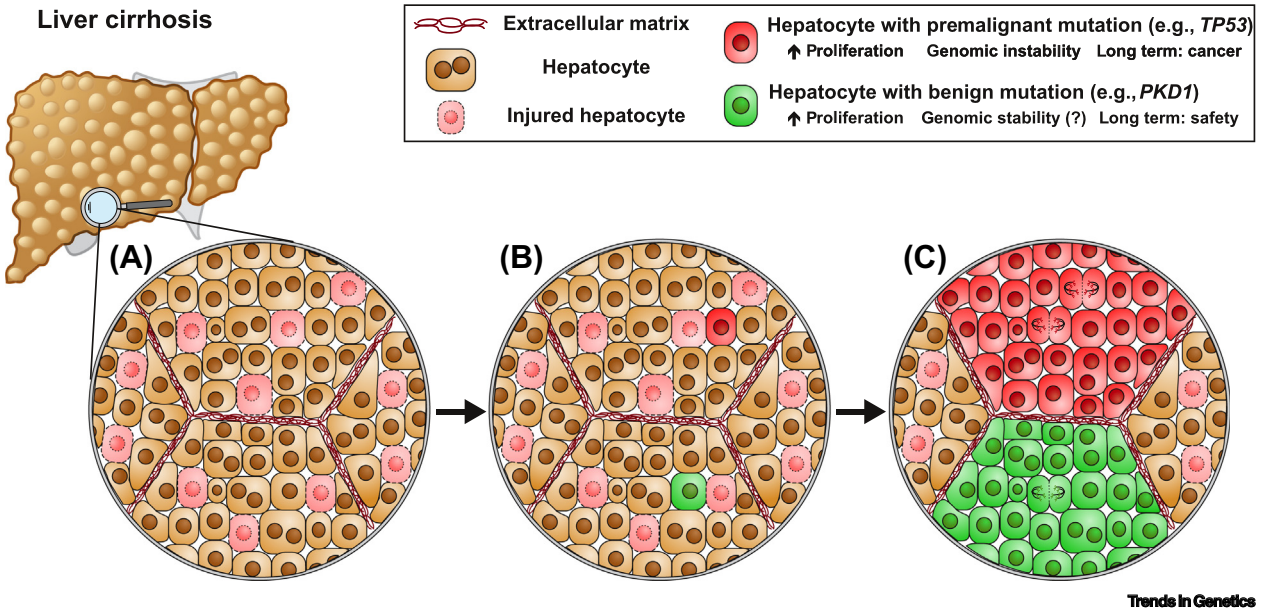

Figure 1. Schematic of Clonal Nodule Formation in Chronic Liver Disease. (A) Chronic liver injury, causing hepatocellular injury (pink), results in fibrosis and nodule formation. (B) In response to injury, some clones gain mutations. (C) Clones with a selective advantage expand to fill the nodule. A recent study supports the concept that, while some clones are premalignant (red), others (green) are not and may protect from injury, improve regeneration, or both [4].

The study by Zhu *et al.* uses elegant methodology to explore the functional role of mutations enriched within the chronically injured liver. They showed that mutations accumulate with disease progression and revealed that, while many of the mutations identified were associated with cancer, some were not. These noncancer-associated mutations could be beneficial for liver regeneration, although their effect upon liver function remains undefined.

The concept of driver mutations providing neoplastic-free disease-resistant regeneration is intriguing. In chronic liver disease, certain areas of the liver, particularly distinct cirrhotic nodules, have markedly different disease involvement. For example, in nonalcoholic fatty liver disease, there may be nodules without fat accumulation, the process that drives damage [5]. Therefore, it is plausible that distinct genetic differences between cirrhotic nodules may underlie these different histological appearances. If mutations could protect from ongoing damage, this would not only promote their expansion, but may also protect them from further genomic instability (Figure 1). A key question is whether distinct forms of injury select for distinct escape mutations driving a disease-specific clonal selection spectrum. This would have implications for diagnosis and therapy. Additionally, will protective clones be sustained if the primary disease is removed and the evolutionary landscape shifts?

A further concept raised by the Zhu *et al.* study is that the liver lobule may act as a natural frontier for hepatocyte expansion, confining clonal outgrowth. This restriction may be formed by fibrotic scars acting as 'bulkheads' to inhibit spread of clonal populations (Figure 1). This suggests that malignant clones are capable of escaping this fibrous constraint and may either push the boundaries to expand the lobule size or, in a more advanced stage, breach this confinement and metastasize. Containment of expanding clones within fibrotic nodules is likely to increase their stiffness, promoting mechanotransduction and activation of pathways, including YAP/TAZ. Interestingly ARID1A is a repressor of YAP/TAZ, circumventing the need for activation through mechanotransduction [6]. Therefore, ARID1A mutations may provide a growth advantage in a softer precirrhotic environment.

Perhaps unsurprisingly, the chromatin modifiers ARID1A and KTM2D, studied by Zhu *et al.*, have been described as altered in human HCC [2]. Given that they are two of the three functionally characterised targets in this report, this is a significant caveat to this study. It will be important to show that other 'protective' mutations described in this study, including PKD1, are not associated with liver tumours in humans, and define the mechanism by which they provide this fail-safe selective advantage.

While the study by Zhu *et al.* raises interesting ideas and advances our knowledge of clonal dynamics in the liver, their work

focuses purely on coding mutations. However, many mutations exist in noncoding regions, particularly promoter regions. Some noncoding region mutations have been a particular discriminator between regenerative nodules and HCC, including TERT [2,7,8]. How noncoding mutations, chromatin remodelling, and dysregulation of translational control differ between regenerative and dysplastic nodules will be of future interest.

Mutations increasing cellular fitness and leading to clonal dominance, particularly in chronic liver disease, have mostly been seen in the light of malignant transformation. However, it now appears that not all of these mutations are harbingers of future cancer. By contrast, some may in fact aid regeneration and protect from further injury. Thus, determining whether this finding also applies across other organ systems will be of significant interest.

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