Beneficial Noncancerous Mutations in Liver Disease

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Abstract:

Chronic liver disease results in fibrosis and cancer. While injury is associated with mutational burden a recent study highlights that not all positively selected mutations in the liver are pre-cancerous. Indeed some may be beneficial to the liver's ability to withstand injury and 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 non-neutral drift in cell populations is seen in health and disease, including cancer. The general consensus is that mutations which are clonally selected within a tissue are inevitably stepping stones towards the development of cancer, and not beneficial to the host.

The liver's role 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 which 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 scientifically but also medically in regenerative medicine, early cancer detection and treatment.

In a recent landmark study Zhu *et al*. dissect the malignant potential of mutations found in non-dysplastic lesions of patients with chronic liver disease by whole exome sequencing, followed by targeted deep sequencing [4]. After carefully excluding malignant tissue and using highly stringent methodology for calling mutations, they were able to identify recurrent mutations occurring in clonal outgrowths in multiple different patients. Intriguingly, it appeared that some of these recurring mutations are not drivers of HCC as they have not been found in previous large scale cancer genomic studies. Using an unbiased *in vivo* CRISPR screen in mice they 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, they 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, they observed clear clonal dominance from these "fitness-promoting mutations" which did not appear to be associated with cancer.

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

The concept of driver mutations providing neoplastic-free disease resistant regeneration is intriguing. In chronic liver disease, it has been noted that certain areas of the liver, particularly distinct cirrhotic nodules, have markedly different disease involvement. For example, in non-alcoholic fatty liver disease, there may be nodules without fat accumulation, the process which drives damage [5]. It is therefore plausible that distinct genetic differences between cirrhotic nodules may underlie these different histological appearances. If mutations could protect from ongoing damage, this would 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 scar acting as 'bulkheads' to inhibit spread of clonal populations (Figure 1). This could suggest 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 [5]. Therefore, ARID1A mutations may provide a growth advantage in a softer pre-cirrhotic environment.

Perhaps unsurprisingly the chromatin modifiers ARID1A and KTM2D, studied by Zhu *et al.* as factors 'not associated with HCC', have been described as altered in human HCC[2]. As 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.

Whilst the study by Zhu *et al.* raises very interesting ideas and advances the knowledge on clonal dynamics in the liver, their work focuses purely on coding mutations. Many mutations, however, exist in non-coding regions, particularly promoter regions. Some non-coding region mutations have been a particularly discriminator between regenerative nodules and HCC, including TERT [2,7,8]. How non-coding mutations, chromatin remodeling and dysregulation of translational control differs between regenerative and dysplastic nodules will 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. On the contrary, some may actually aid regeneration and protect from further injury. Determining whether this finding applies widely across other organ systems will be of interest.

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Fig.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].