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Hepatic Oncology

Expert panel: Thomas Tu, Sydney Medical School, The University of Sydney, NSW, Australia and Centenary Institute, Sydney, NSW Australia; Nicholas Shackel, Sydney Medical School, The University of Sydney, NSW, Australia and Centenary Institute, Sydney, NSW Australia and A W Morrow Gastroenterology & Liver Centre, Royal Prince Alfred Hospital, Camperdown, NSW, Australia; Geoffrey McCaughan, Sydney Medical School, The University of Sydney, NSW, Australia and Centenary Institute, Sydney, NSW Australia and A W Morrow Gastroenterology & Liver Centre, Royal Prince Alfred Hospital, Camperdown, NSW, Australia.

Nakagawa H, Umemura A, Taniguchi K *et al.* ER stress cooperates with hyper-nutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* 26(3), 331–343 (2014).

Obesity-driven non-alcoholic fatty liver disease progresses to non-alcoholic steatohepatitis (NASH) and can lead to hepatocellular carcinoma (HCC). Endoplasmic reticulum (ER) stress has been known to occur with premalignant liver diseases, especially in NASH, though its causative role in HCC development has not been previously demonstrated. Due to the rising incidence of NASH-associated HCC, characterizing the factors that may be driving hepatocarcinogenesis (e.g., ER stress) is needed to develop novel treatments.

To explore the role of ER stress in NASH-associated HCC, Nakagawa *et al.* have used a mouse model expressing *uPA* under a hepatocyte-specific promoter, resulting in transient intra-hepatic ER stress. Wild-type and *uPA* mice were fed a high-fat diet from 6 weeks of age and were monitored up to 40 weeks.

After 32 weeks, mice with ER stress (*uPA* mice) showed small HCCs and adenomas, in which there was increased expression of classical HCC-associated proteins (STAT3, ERK, JNK and EpCAM). Adding a high-fat diet to mice with ER stress further increased HCC size. Histology of these mice revealed typical features of NASH (e.g., hepatocyte ballooning and bridging fibrosis) eventually cumulating in HCC. TNF pathway activity in both the HCC tumor and liver macrophages was necessary for NASH pathogenesis as well as the enhanced HCC progression associated with a high-fat diet.

This comprehensive paper highlights the role of ER stress as an active driver of HCC. Additionally it also found that TNF is an important mediator of NASH and NASH-associated HCC. This suggests that blocking the TNF pathway is a potential treatment in the future.

Nault JC, Calderaro J, Tommaso LD *et al.* TERT promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. *Hepatology* doi:10.1002/hep.27372 (2014) (Epub ahead of print).

The difficulty of detecting hepatocellular carcinoma (HCC) at an early stage remains a major contributor to its associated high mortality. Genetic determinants of the early steps of HCC carcinogenesis are poorly characterized. Understanding the early genetic changes in HCC development may help identify individuals at risk of HCC.

To address this deficiency, Nault *et al.* analyzed DNA mutations in 96 liver nodules from 58 cirrhotic patients. From the consensus pathology reports of six experts, each nodule was classed into low- or high-grade dysplastic nodule, early HCC, and small and progressed HCC. Sanger sequencing was used to detect DNA mutations in the *TERT* gene promoter and ten recurrently mutated genes in HCC, including *CTNNB1*, *TP53* and *ARID1A*.

Progressive mutation of the *TERT* promoter was seen in progression towards liver cancer, with 6, 19, 61 and 42% of low-grade dysplastic nodule, high-grade dysplastic nodule, early HCC, and small and progressed HCC containing mutations

respectively. However, mutations in the ten recognized HCC-associated genes studied were only detected in small and progressed HCC with no single change being seen in all cases.

This study shows that *TERT* promoter mutation is an early change occurring before HCC becomes apparent, offering a potential target for early detection. However, these results also highlight the inter-tumoural heterogeneity in HCC, which suggests that an accurate and sensitive biomarker for early detection is still a distant goal.

Jhunjunwala S, Jiang Z, Stawiski EW *et al.* Diverse modes of genomic alterations in hepatocellular carcinoma. *Genome Biol.* 15(8), 436 (2014).

To find genetic biomarkers in the heterogeneous landscape of hepatocellular carcinoma (HCC) requires a broad-scale approach. Thus Jhunjunwala *et al.* conducted one of the most comprehensive next-generation sequencing studies to date on HCC samples. The exomes of tumors and adjacent non-tumor tissue of 30 patients were sequenced. Further, the whole genome and transcriptome was sequenced using RNA-seq for an additional 12 patients.

Analysis of the next-generation sequencing data found 13 of the 42 patients had mutations and structural variations in genes known to be associated with HCC (*AXIN1*, *CTNNB1* and *TP53*). Mutations in the *TERT* gene promoter (detected in four patients) were directly associated with upregulation of the corresponding mRNA expression. In these patients, a possible biomarker *LAMA2* was observed to be mutated and down-regulated in six of 42 HCC tumors. Analysis of a publicly available microarray expression data set of 300 HCC patients (generated by the University of Hong Kong) showed that lower than average *LAMA2* expression was significantly associated with poorer overall survival and 30% increased risk of HCC recurrence.

Future prospective studies are required to determine the clinical usefulness of *LAMA2* expression. This study has emphasized the highly heterogeneous genomic changes of HCC, which need to be incorporated into the future research of novel biomarkers.

Powers TW, Neely BA, Shao Y, *et al.* MALDI imaging mass spectrometry profiling of N-glycans in formalin-fixed paraffin embedded clinical tissue blocks and tissue microarrays. *PLoS ONE*

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HCC is not only associated with inter-tumor heterogeneity, but also intra-tumor heterogeneity, with several cell types and cell phenotypes being present within the same tumor. There is an important distinction and interaction between the tumor cells and the supporting stroma, which is responsible for aspects of the tumor biology such as angiogenesis. Immunohistochemistry is the typical method of analyzing tissue expression of proteins, but is constrained by a relatively low throughput for comparatively few targets and is unsuited to detect post-translational modifications, such as glycosylation. In this study, Powers *et al.* used matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) to simultaneously identify, detect and quantify hundreds of N-glycosylated proteins on formalin-fixed paraffin-embedded liver tissue *in situ*.

Initially, this group used commercially-available tissue microarray of 16 HCC and matched non-tumor formalin-fixed paraffin-embedded tissues incubated with PNGaseF to digest and release N-glycosylated protein fragments. Then, MALDI-IMS was used to produce a mass spectrum at 200 μm intervals on the tissue. A 'map' was generated for each mass peak and used to differentiate tumor from non-tumor tissues. A total of 176 glycoprotein-associated peaks were detected in the HCC tissues with ~50% of the peaks being significantly upregulated in tumor tissues. Impressively, 61 of the identified peaks performed well as potential diagnostic markers (all with AuROC >0.80).

Despite the small sample set, this paper highlights the potential largely unrecognized role of MALDI-IMS in HCC diagnosis. Simultaneously detecting and quantifying hundreds of proteins *in situ* will likely account for the known heterogeneity of HCC (compared with studying just one protein using immunohistochemistry).

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