Abstract: 11S

Single nucleus RNA sequencing of pre-malignant liver to predict NASH-driven liver cancer.

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Background and Aim: Current approaches to stage chronic liver diseases have limited utility to directly predict hepatocellular carcinoma (HCC) risk. The consequence is lack of appropriate surveillance, late diagnosis, and poor survival of HCC patients. Thus, development of new tissue and blood biomarkers is urgently needed. **Methods:** We employed bulk RNA sequencing and single nucleus RNA sequencing (snRNA-seq) to assess the cellular microenvironment of healthy and chronically injured pre-malignant livers using three well-characterised HCC mouse models: (a) choline-deficient, ethionine-supplemented diet - CDE, (b) thioacetamide supplementation - TAA, and (c) major urinary protein (MUP)-urokinase-type plasminogen activator (uPA) mice on a high-fat diet - MUP-uPA. In addition, various specific regions of interest in 3-week (injury induction) and 6-month (established tumours) TAA tissue were micro-dissected and assessed by whole genome amplification and low-pass whole genome sequencing to evaluate their respective mutational burden. For all animal experiments, mice were anaesthetised by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). **Results:** This analysis identified a plethora of normal and disease-associated cell types and subsets and unraveled a novel disease-associated hepatocyte transcriptional state (daHep). These cells were absent in healthy livers but were increasingly prevalent as chronic liver disease progressed towards hepatocarcinogenesis. Gene expression deconvolution of 1,439 human liver transcriptomes from publicly available datasets revealed that daHep frequencies highly correlate with current histopathological liver disease staging systems. Importantly, data obtained from liver biopsies of high fat diet-fed MUP-uPA mice with established non-alcoholic steatohepatitis prior to any signs of tumorigenesis predicted future HCC development in this model of partial HCC penetrance, confirming the daHep signature as a novel HCC-prognostic marker. Finally, recently obtained low-pass whole genome sequencing data of micro-dissected healthy hepatocytes, daHep and HCC revealed that daHep share a mutational burden profile with HCC even at the early time point of 3 weeks, while healthy hepatocytes at the 6-month time point where HCC is well-established still display a normal genetic phenotype. **Conclusion**: This novel transcriptional signature with diagnostic and, more importantly, prognostic significance has the potential to change the way chronic liver disease patients are staged, surveilled and risk-stratified.

Professor Nina Tirnitz-Parker is the Head of the Liver Cancer Program at the Curtin Health Innovation Research Institute and Co-Director of the Liver Cancer Collaborative (LCC, www.livercancerwa.org.au) - a multi-disciplinary consortium of hepatologists, oncologists, interventional radiologists, computational biologists and cancer researchers. The LCC is establishing a comprehensive tissue and blood biobank of liver disease patients at different stages of disease and cancer. Patient-matched analyses are performed using multi-omics approaches and organoid drug screening, with the ultimate goal of generating a publicly accessible database for patient and treatment outcome predictions towards personalised precision liver cancer treatment.