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EDITORIAL

Single-Cell and Bulk Transcriptome Profiling Reveals Unique Features of Diploid and Polyploid Hepatocytes

Most mammalian somatic cells are diploid and contain pairs of each chromosome, but there are also polyploid cells with additional sets of chromosomes. Hepatocytes are among the best described polyploid cells, with polyploids comprising more than 25% and 90% of the hepatocyte population in human beings and mice, respectively. Polyploidization begins during postnatal development in rodents when proliferating diploid hepatocytes fail to complete cytokinesis and produce binucleate tetraploid daughter cells containing 2 diploid nuclei. These binucleate tetraploid hepatocytes enter the cell cycle, and after nuclear envelope dissolution, replicate their DNA, which then is distributed to a pair of mononucleate tetraploid daughter cells during cytokinesis. The tetraploid daughters then can generate octaploid and higher ploidy hepatocytes during subsequent rounds of mitosis. Hepatocyte function is influenced, at least in part, by location within the liver lobule, and there are conflicting reports on the geographic location of ploidy subpopulations. Although hepatic chromosomal variations were documented more than a century ago, the role played by these cells in liver homeostasis, regeneration, and disease has been poorly understood. Recently, several studies have advanced our thinking about chromosomal variations in the liver. For example, Zhang et al[1] showed that polyploid hepatocytes protect against liver cancer by providing a "buffer" against tumor-suppressor loss. Inactivation of 1 tumor suppressor copy in a diploid cell led to loss of heterozygosity and increased potential for transformation, while polyploid hepatocytes contained additional chromosome sets (effectively providing back-up tumor-suppressor copies) and were protected from transformation. Our group found that in response to proliferative stimuli, diploid hepatocytes had faster cell-cycle entry and progression than polyploids, suggesting that diploid hepatocytes have a proliferative advantage and are immediate drivers of liver regeneration.[2] Thus, the liver field is only beginning to understand how ploidy variations affect function, illustrating the need for additional studies to understand how ploidy affects liver homeostasis and disease.

A major unanswered question is whether there are basal differences between diploid and polyploid hepatocytes. Katsuda et al[3] tackle this question by identifying gene expression patterns by these populations. Hepatocytes from adult rats were separated by ploidy and pools of hepatocytes were subjected to either bulk microarray analysis or single-cell quantitative reverse-transcription polymerase chain reaction using a custom panel of 47 genes (diploid cells, n = 90; tetraploid cells, n = 245). Most genes associated with liver function were expressed similarly by ploidy subpopulations, but unique gene signatures emerged. First, genes associated with zone 3 expression (eg, *Glul*, *Cyp7a1*, *Slc1a2*) were enriched in diploid hepatocytes, whereas zone 1 genes (eg, *Alb*, *G6pc*, *Tat*) were more highly expressed by polyploids. The data suggest that diploid and polyploid hepatocytes preferentially localize to the pericentral and periportal areas, respectively. Second, genes associated with cell-cycle progression were expressed by polyploid hepatocytes, particularly octaploids (eg, *Ccna2*, *Ccnb2*, *Mki67*). This observation was surprising because polyploid hepatocytes recently were associated with a reduced or delayed proliferative capacity.[2] Third, microarrays showed that diploid hepatocytes were enriched with genes associated with progenitor cell phenotype. Furthermore, single-cell quantitative reverse-transcription polymerase chain reaction showed that progenitor marker expression was heterogeneous, with $Axin2^{+}$ $Prom1^{+}$ $Lgr5^{+}$ "triple positive" hepatocytes accounting for 12% of diploid cells. In summary, diploid and polyploid hepatocytes appear to localize preferentially to distinct regions within the liver lobule, and quiescent ploidy subpopulations have defined characteristics. These conclusions contrast with a 2007 report that found few gene expression differences between mouse diploid, tetraploid, and octaploid hepatocytes analyzed in bulk using microarray methods.[4] Technical differences likely explain the divergent conclusions by each study, which underscores the utility of revisiting old questions with newer techniques and improved sensitivity.

Katsuda et al[3] looked at diploid and polyploid hepatocytes and provided new insights into hepatocyte biology, which raises important questions for future work. Transcriptomic profiling was performed using adult rat hepatocytes. Are the findings applicable to mice where functional data exist, and, more importantly, to human beings? Gene profiling was performed in quiescent cells, and it remains to be seen how expression is altered in proliferating or injured subpopulations. Diploid hepatocytes were found to harbor a subset of cells with progenitor characteristics. Are these cells hepatocytes, or do they represent an entirely new population? Functional testing of prospectively isolated populations is essential. Finally, this work includes a transcriptomic analysis of diploid and polyploid hepatocytes at the single-cell level. However, it is important to recognize that low numbers of cells were investigated with a limited set of genes. It therefore is necessary to verify and extend the findings using single-cell RNA sequencing, which will permit transcriptomic analysis of thousands of sorted cells

and thousands of gene targets. Such an unbiased analysis may show unique cell types within diploid and polyploid subpopulations and further illuminate functional properties, structural features, and cellular hierarchies within the liver.

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References

1. Zhang S, Zhou K, Luo X, et al. The polyploid state plays a tumor-suppressive role in the liver. Dev Cell 2018;44:447–459.
2. Wilkinson PD, Delgado ER, Alencastro F, et al. The polyploid state restricts hepatocyte proliferation and liver regeneration in mice. Hepatology 2019;69:1242–1258.
3. Katsuda T, Hosaka K, Matsuzaki J, Usuba W, Prieto-Vila M, Yamaguchi T, Tsuchiya A, Terai S, Ochiya T. Transcriptomic dissection of hepatocyte heterogeneity: linking ploidy, zonation, and stem/progenitor cell characteristics. Cell Mol Gastroenterol Hepatol 2020;9:161–183.
4. Lu P, Prost S, Caldwell H, et al. Microarray analysis of gene expression of mouse hepatocytes of different ploidy. Mamm Genome 2007;18:617–626.

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