LIVER

New insight into hepatocyte genetic diversity and adaptation to injury

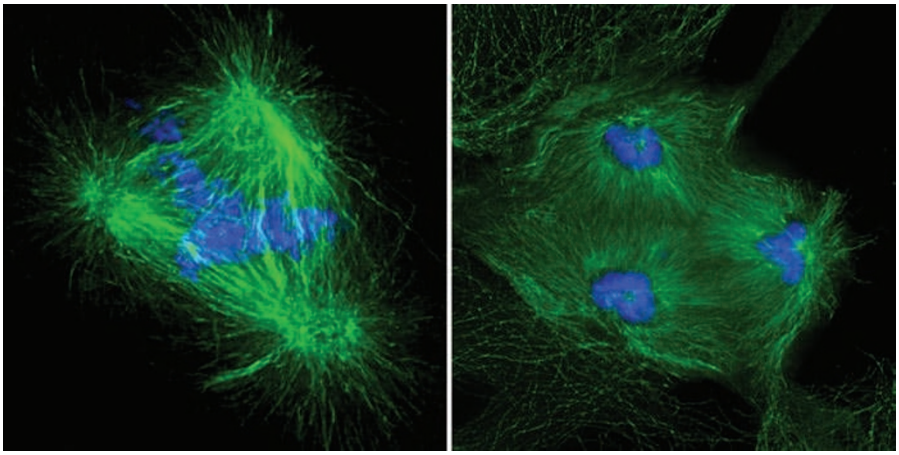
A new phenomenon, called the 'ploidy conveyor' allows hepatocyes to become genetically diverse and enables them to adapt to injury, suggest Andrew Duncan, Markus Grompe and colleagues in a recently published *Nature* paper.

All mammalian species are known to have hepatocyes that contain more than the usual paired set of chromosomes, but until recently such polyploid hepatocytes were thought to be terminally differentiated and not able to proliferate. Evidence also indicates that hepatocyte polyploidy does not function to enhance their metabolic capacity, as previously suggested. Duncan and colleagues were, therefore, motivated to understand the functional purpose of polyploidization.

Prior work by the group had shown rare hepatocytes derived by fusion of blood cells and hepatocytes were aneuploid (that is, had numerical chromosomal gains and/or losses) and capable of ploidy reversal. "Based on these findings, we hypothesized that normal polyploid hepatocytes were aneuploid and could undergo ploidy reversal," explains Duncan.

The researchers used multiple approaches to find out whether their hypothesis was correct. Fluorescence-activated cell sorting (FACS) was used to isolate ploidy hepatocyte populations from male mice, with the DNA content of a cell being reflected by the amount of fluorescence in that cell—tetraploid (4*n*) cells fluoresced twice as much as diploid (2*n*) cells and octaploid (8*n*) cells twice as much as tetraploid cells.

Octaploid hepatocytes were transplanted into female mice and allowed to repopulate the liver; hepatocytes harvested from the repopulated livers of the mice were then analyzed by FACS and cytogenetic karyotyping to determine the degree of chromosome loss or gain. Donor-derived hepatocytes were confirmed to have reduced ploidy and most were aneuploid, suggesting to Duncan and colleagues that most normal polyploid hepatocytes utilise ploidy reversal when forced to undergo extensive cell division.



A tetraploid hepatocyte with multipolar spindles in metaphase (left) and undergoing multipolar mitosis, with three daughter nuclei shown in telophase (right). Microtubules (green); DNA (blue). Courtesy of A. Duncan and M. Grompe.

Similarly, octaploid hepatocytes were grown in cell culture and the cell ploidy determined after 5 days (that is, 1–2 cell cycles). Again, the ploidy of the daughter hepatocyes was reduced, which indicated that ploidy reversal occurred via a single-step process.

Imaging of fixed and live cells was also performed to see whether polyploid hepatocytes underwent multipolar cell division as a means of producing aneuploidy. Most polyploid hepatocytes were found to undergo normal bipolar cell division, but completely surprising was the finding that ~4% of polyploid hepatocytes underwent multipolar cell division, giving rise to three or more nonidentical aneuploid daughter cells. Multipolar cell division has previously been described only in cancer cells, which generally die after the process is completed. The finding that aneuploidy in the liver is pervasive was also surprising because aneuploidy is typically associated with cancer.

"Together our data show that hepatocyte proliferation involves a cycle of polyploidization, ploidy reversal and aneuploidy. We call this dynamic process the 'ploidy conveyor'," says Duncan.

So, what are the implications of these findings? "We suggest that proliferating hepatocytes polyploidize and undergo ploidy reversal to specifically generate unique hepatocytes with different mixtures of chromosomes," explains Duncan. "This genetic diversity may operate as an adaptive mechanism, serving as a substrate for selection of those hepatocytes most resistant to xenobiotic or nutritional injury."

The researchers are now studying the effect of different types of liver injury on hepatocytes and hypothesize that an injury-specific and unique 'karyotypic signature' could be present in the 'fittest' hepatocytes.

"We believe aneuploidy is a normal characteristic of hepatocytes and may not necessarily be a predisposition to cancer," concludes Duncan, who is also working to characterize aneuploidy in normal human and rodent cells, including hepatocytes.

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