Hepatocyte ploidy modulation in liver cancer

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Polyploidy, a balanced amplification of the genome, is common in the liver. The function of hepatic polyploidy is not entirely clear, but growing evidence shows that polyploidy can protect the liver from tumor formation. In this issue of *EMBO Reports*, Sladky and colleagues identify the PIDDosome as a ploidy sensor that regulates liver cancer (Sladky et al, 2020b).

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See also: VC Sladky et al (December 2020)

Hepatocytes, the primary functional cells of the liver, are characterized by variations in DNA content (Donne et al, 2020). Hepatocytes are exclusively diploid at birth and, during postnatal development, undergo rounds of acytokinetic and cytokinetic mitosis to generate polyploid daughters with additional chromosome sets (tetraploid, 4n; octaploid, 8n; or higher). Diploid (2n) hepatocytes have a single nucleus with 2n nuclear content, while polyploid hepatocytes are either mononucleate (with a single 4n, 8n, or 16 nucleus) or binucleate (with pairs of 2n, 4n, or 8n nuclei). Polyploid hepatocytes comprise a major fraction of healthy adult livers, accounting for 25–50% of hepatocytes in humans and more than 90% in mice.

Polyploidy is frequently associated with chromosomal instability and cellular dysfunction that may contribute to cancer growth (Lens & Medema, 2019). This is not necessarily the case in the liver where the incidence of spontaneous cancer is low, and polyploidy accompanies normal function and homeostasis. In fact, polyploid hepatocytes have been shown to safeguard the liver from hepatocellular carcinoma (HCC). Proof-of-concept studies investigated HCC formation using genetic models of low ploidy and hyperploidy (Kent et al, 2016; Zhang et al, 2018; Wilkinson et al, 2019; Lin et al, 2020). Mice with hyperploidy livers developed few, if any tumors; wild-type (WT) mice with physiological levels of polyploidy developed modest numbers of tumors; and mice with low polyploidy developed extensive tumor burden with rapid kinetics. The polyploid state has been shown to influence HCC by several mechanisms—providing protection from tumorigenesis with extra copies of tumor suppressor genes and by restricting hepatocyte proliferation—but many unknowns still remain (Zhang et al, 2018; Wilkinson et al, 2019). In this issue of *EMBO Reports*, Sladky and colleagues investigate molecular mechanisms linking polyploidy to oncogenic proliferation; they demonstrate a critical role for the PIDDosome ploidy surveillance pathway in HCC initiation and expansion (Sladky et al, 2020b).

The PIDDosome is a protein complex, comprised of PIDD1, RAIDD, and CASP2, that functions as a molecular sensor of supernumerary centrosomes to induce p53-mediated cell cycle arrest (Fava et al, 2017). PIDD1 localizes to the mother centriole during a traditional cell cycle, and when extra centrosomes are formed—as happens when polyploid hepatocytes arise from acytokinetic mitosis—RAIDD and CASP2 are recruited to PIDD1. Once the PIDDosome is assembled, CASP2 is activated and cleaves the E3 ubiquitin ligase MDM2, which permits p53 stabilization and p21-mediated cell cycle arrest. Recently, Sladky and co-workers demonstrated a critical role for the PIDDosome in regulation of hepatic polyploidy (Sladky et al, 2020a). Livers of adult WT mice contained a standard ploidy spectrum with more than 90% polyploidy, and within the polyploid fraction, most hepatocytes were 4n. Germline deletion of PIDDsome proteins disrupted the ploidy spectrum where overall polyploidy remained greater than 90%, but 4n hepatocytes were reduced, while 8n and 16n hepatocytes were dramatically increased. Hence, PIDDsome activity keeps polyploidy levels within “normal” limits and restricts hyperpolyploidization.

In the current work, the authors focused on the PIDDsome and liver cancer. Using the diethylnitrosamine (DEN) model, WT and PIDDsome-deficient mice (Pidd1+/−, Raidd+/−, or Casp2−/−) were injected with DEN on postnatal day 15 to induce tumor formation (Sladky et al, 2020b). By 10 months, PIDDsome knockouts formed tumors, but the tumors were smaller and less numerous compared to WT (Fig 1A). Tumors in each mouse shared similar characteristics, including increased numbers of cycling cells, reduced hepatic polyploidy, and equivalent low-level aneuploidy. An opposite trend was seen in non-tumor liver tissue where most hepatocytes are highly polyploidy and quiescent. Thus, PIDDsome knockout mice with high-degree polyploidy are protected from hepatocarcinogenesis, and tumor progression is driven by lower-ploidy hepatocytes.

Next, the authors turned to individual members of the PIDDsome complex. In both WT mice and humans, HCCs were broadly characterized by increased CASP2 and PIDD1 protein and/or mRNA, compared to healthy liver (Sladky et al, 2020b). In humans, elevated CASP2 and PIDD1 correlated with hallmark pathways associated with proliferation (Fig 1B). This result is not entirely surprising since the authors...
previously demonstrated binding sites of E2F1, a transcription factor and potent driver of the cell cycle, in promoters of CASP2 and PIDD1. The data suggest that CASP2 and PIDD1 expression is a secondary effect of tumor cell proliferation. Indeed, recurrence-free survival was markedly reduced in patients with high CASP2 compared to patients with low expression, underscoring the severity of disease associated with CASP2 elevation.

Finally, Sladky et al examined whether hepatic polyploidy correlated with HCC progression in patients. They developed a simple readout of ploidy spectrum based on cell size: Lower-ploidy hepatocytes are small and found at high density in liver, while polyploid hepatocytes are large and found at lower density (Sladky et al., 2020b). Using a morphometric approach, HCCs from 223 patients of various etiologies were stratified into groups based on cell density. Remarkably, patients with tumors of low cell density (interpreted as highly polyploid) had significantly improved recurrence-free survival, suggesting that polyploidy attenuates hepatocarcinogenesis in humans.

This new work has important implications for liver cancer. First, in mouse models, high-degree polyploidy induced by PIDDosome deficiency protects the liver from tumorigenesis. The data support a model where tumor initiation occurs preferentially in lower-ploidy hepatocytes, but not higher-ploidy hepatocytes (either pre-existing hepatocytes or those formed even after mutagenesis). Second, CASP2 and PIDD1 levels increase during tumor progression, and this is driven by pro-proliferation signals (notably E2F1) and is likely independent of PIDDosome activity. Elevated CASP2 expression correlated with reduced survival in patients, suggesting that CASP2 could potentially be used as a prognostic marker of disease severity. Third, morphometric imaging approaches have been used successfully to directly assess hepatocyte nuclear content in liver sections. Here, using a streamlined approach based on hepatocellular density, patients with low cell density tumors had improved recurrence-free survival, indicating hepatic polyploidy is protective in human HCC. These results contrast with a separate study showing the most aggressive HCCs are enriched with highly polyploid hepatocytes, particularly HCCs with mutated p53 (Bou-Nader et al., 2020). Both observations are probably correct as ploidy-dependent effects may be context dependent, which highlights the need for further investigation. Fourth, considering that healthy human livers are 25-50% polyploid, it is tempting to speculate whether induced polyploidy is a viable strategy to prevent HCC initiation or slow HCC progression (Lin et al., 2020). Selective deletion of PIDDosome proteins, such as CASP2, may be ideal targets for “therapeutic polyploidization”. In conclusion, the idea that liver polyploidy restricts HCC development is new and understanding molecular mechanisms that link polyploidy and tumorigenesis (like the
PIDDosome) is critical for development of patient diagnostics or therapies.

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