Emergence of cancer stem cells in hepatocellular carcinoma

Sahra Pilz & Gunnar Schotta

Liver cancer represents the second most deadly human malignancy. The major histological subtype called hepatocellular carcinoma (HCC) arises by chronic inflammation-triggered regenerative responses of normally quiescent hepatocytes and progenitors, respectively. Such regenerative stress accelerates the accumulation of genetic and epigenetic changes (Yamashita & Wang, 2013), while detailed mechanisms remain uncertain. In this issue of The EMBO Journal, Nikolaou et al present a novel HCC model that facilitates both isolation and molecular characterization of self-renewing, HCC-propagating cancer stem cells that could instruct future interventions (Nikolaou et al, 2014).

The experiments conducted by Nikolaou et al established a new model for HCC addressing this gap and facilitating direct isolation of CSCs. They functionally analyzed the histone methyltransferase PRSET7 in liver development. PR-SET7 is the sole enzyme that catalyzes histone H4K20 dimethylation (H4K20me2). This methylation plays an important role in many cellular processes like DNA replication, DNA damage response and mitotic condensation (Jørgensen et al, 2013). PR-SET7 knock-out (ko) is lethal in mice and flies and leads to cell cycle arrest and apoptosis (Driskell et al, 2012; Jørgensen et al, 2013). Using a cre-lox system, Nikolaou et al knocked out PR-SET7 in embryonic mouse livers. Consistent with the essential role of PR-SET7, prenatal lethality was observed. E18.5 embryos were anemic and had a severely reduced liver volume. In contrast, mice with PR-SET7 deletion in adult liver survived and only displayed areas of necrotic cell death. Necrosis was found in regenerating areas of the liver and was caused by the attempted proliferation of hepatocytes which die due to the lack of PR-SET7. To stimulate liver regeneration, the authors decided to perform partial hepatectomy (PHx), which leads to compensatory growth of the remaining tissue by proliferation of adult hepatocytes (Fig 1). PR-SET7-deficient cells die upon proliferation, and enhanced necrotic areas in PR-SET7-deficient PHx livers coincided with chronic inflammation. Interestingly, all PR-SET7 ko mice with PHx developed HCC between postnatal days 240–300. Thus, PHx in PR-SET7 ko livers reflects the inflammatory aspect of human HCC, although in humans hepatocyte proliferation is not as severely impaired.

Remarkably, the tumor cells isolated by Nikolaou et al showed CSC properties: they could be cultured in vitro over several passages and they could give rise to tumors with characteristics of the parental tumor when engrafted into immunodeficient mice. These properties distinguish them from a previously described HCC model which was only transplantable into already damaged livers (He et al, 2013). Why the tumor progenitor cells isolated by He et al and the CSCs isolated by Nikolaou et al differ, is currently unclear. One possibility is that they might stem from different origins. The inability of PR-SET7-deficient hepatocytes to...
The histone methyltransferase Setd8 acts in concert with c-Myc and is required to maintain skin. *EMBO J* 31: 616–629


**Figure 1. PHx of adult PR-SET7 ko livers leads to HCC onset.**

PHx in PR-SET7-deficient livers causes proliferation of normally quiescent hepatocytes. Proliferation of PR-SET7-deficient hepatocytes leads to necrotic cell death which is proceeded by an inflammatory response. Thus, liver regeneration involves differentiation of initially PR-SET7-proficient HPCs to hepatocytes. Excessive proliferation of HPCs in combination with chronic inflammation results in the development of HCC. A remarkable feature of these tumors is the presence of CSCs with stemness properties: they can self-renew and they can be transplanted into immunodeficient mice, where they give rise to new tumors.

In summary, Nikolaou et al created a new and very informative HCC model. Arising HCCs present with very high penetrance and surprisingly little tumor heterogeneity. Notably, the tumor cell population includes CSCs that have the ability to self-renew and to initiate tumors when transplanted into immunodeficient mice. Although these cells have been phenotypically and functionally well characterized, we still know very little about their genetic and epigenetic aberrations. Further analyses should reveal CSC-specific oncogenes and tumor suppressor genes. Furthermore, the high penetrance of CSCs in this tumor model will allow for a better understanding of their biological features such as the regulation of proliferation and drivers for their metastatic capacity. Even more intriguing will be potential translation of these animal studies for human HCCs.

**References**