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INSIGHTS

Iron overload and liver cancer

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In this issue of *JEM*, Muto et al. (https://doi.org/10.1084/jem.20180900) generate a novel mouse model of liver cancer induced by iron overload by deleting the iron-sensing ubiquitin ligase FBXL5 specifically in hepatocytes and exposure to a chemical carcinogen.

Hereditary hemochromatosis (HH) is an important risk factor for the development of hepatocellular carcinoma (HCC), one of the most frequent and devastating types of cancer (El-Serag, 2011). HH is characterized by excessive absorption of iron, which is accumulated in different organs, including the liver, inducing toxicity (Pietrangelo, 2010). As a consequence, HH patients are at increased risk of developing liver fibrosis, cirrhosis, and eventually HCC. It has been proposed that iron accumulation in hepatocytes promotes oxidative stress, cell death, and compensatory proliferation, which all together favor the accumulation of mutations in hepatocytes and subsequent malignant transformation (Fu and Chung, 2018). HH is in general caused by homozygous mutations in the homeostatic iron regulator (*HFE*) gene, which is involved in the tight regulation of iron absorption. Iron homeostasis is also regulated by F-box and leucine-rich repeat protein 5 (FBXL5) and iron regulatory protein 2 (IRP2; Moroishi et al., 2011). IRP2 is an RNA-binding protein that controls the production of proteins involved in iron accumulation while FBXL5 negatively regulates IRP2. While the increased risk of HCC development in patients with excessive iron accumulation is well established, the basic and translation aspects are understudied, in part due to the lack of faithful genetically engineered mouse models recapitulating iron overload and HCC development. In this study by Muto et al., a genetically engineered mouse model of HCC characterized by iron overload has been developed.

To study the effect that iron overload can have in liver tumorigenesis, Muto et al. (2019) generated a genetically engineered mouse model in which *Fbxl5* is specifically deleted in hepatocytes, a model that spontaneously develops steatohepatitis but not liver tumors (Moroishi et al., 2011). To generate liver tumors in the background of liver damage by iron overload, mice were treated with the chemical carcinogen diethylnitrosamine (DEN). *Fbxl5* deletion in hepatocytes combined with DEN treatment led to tumor acceleration and reduced survival and was accompanied by iron accumulation. Mechanistically, *Fbxl5* deletion led to Irp2 accumulation, and *Irp2* deletion was able to rescue the increased tumorigenesis caused by lack of *Fbxl5*, demonstrating the direct role that Irp2 plays in iron-mediated HCC. Fbxl5 deficiency led to iron accumulation in hepatocytes and subsequent oxidative stress and inflammation, which in turn promoted the compensatory proliferation of hepatocytes and the development of tumors. In fact, Fbxl5-deficient tumors acquired more somatic mutations after treatment with the chemical carcinogen than control mice under the same treatment, confirming that iron overload is associated with higher mutational load. Furthermore, Fbxl5 deletion not only cooperated with the chemical carcinogen DEN but also with overexpression of the hepatitis C virus core antigen, which also led to the development of HCC. In summary, the damage caused by iron overload increases the predisposition to develop HCC in combination with a chemical carcinogen or viral infection in mice.

In HCC patient samples from five different cohorts—The Cancer Genome Atlas (TCGA) Liver Hepatocellular Carcinoma (LIHC) dataset (Cancer Genome Atlas Research Network, 2017), the Gene Expression Omnibus database (Roessler et al., 2010), and three datasets from the Array Express archive of the European Bioinformatics Institute—there was a significant association between low FBXL5 expression levels and reduced survival. Interestingly, in the TCGA-LIHC dataset, patients with low *FBXL5* mRNA levels presented more somatic mutations than patients with high *FBXL5* mRNA levels, confirming the results obtained in the in vivo mouse model, which further validates the utility of the novel mouse model to recapitulate HCC that is associated with *FBXL5* loss in patient samples. Finally, analysis of IRP2 protein levels in an independent patient cohort of 22 HCC patients collected by the authors demonstrated that high protein levels of IRP2 were also associated with worse prognosis.

Insights from Pedro Molina-Sánchez and Amaia Lujambio.

The generation of this novel mouse model of HCC driven by iron overload is likely to have a great impact in the understanding of the role of iron homeostasis in liver cancer development. One direct application will be testing how additional risk factors of HCC cooperate with iron overload to induce liver cancer. In the current study by Muto et al.

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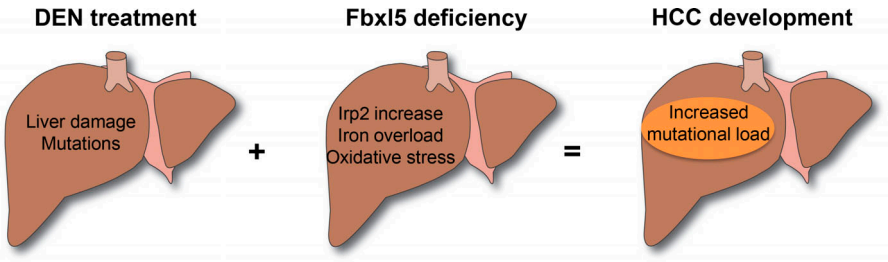

Schematic of the novel mouse model of HCC driven by iron overload. The combination of DEN treatment (which induces mutations) with Fbxl5 deficiency (which leads to Irp2 overexpression, iron overload, and oxidative stress) gives rise to liver tumors with high mutational load.

(2019), it has been demonstrated that iron overload cooperates with chemical carcinogenesis and overexpression of the hepatitis C virus core antigen. For example, it will be very interesting to combine iron overload, driven by Fbxl5 deficiency, with liver damage driven by the fibrogenic agent carbon tetrachloride (CCl_4), high-fat diet, or alcohol abuse. A second application will be to harness the model of iron overload–driven HCC to identify potential therapeutic strategies that could be used in HH patients with HCC or HCC patients with low levels of FBXL5. In principle, strategies that reduce oxidative stress combined with reduction of iron absorption could help prevent or delay liver tumorigenesis in patients at risk. One intriguing possibility will be to test the potential of immune checkpoint inhibitors in this model. Tumor mutational load is a critical factor that predicts response to immune checkpoint inhibitors targeting CTLA-4 (cytotoxic T lymphocyte–associated protein 4) or PD-1 (programmed cell death protein 1; Samstein et al., 2019). Since tumor samples with FBXL5 deficiency present higher mutational burden than samples with higher FBXL5 expression levels in both murine and human tumors, immune checkpoint inhibitors could potentially be successful. However, something to consider is that iron overload can also occur in the context of deficiency of β-2-microglobulin (de Sousa et al., 1994), which is part of the MHC class I and is therefore required for proper antigen presentation and response to immunotherapies. Finally, sequencing and characterization of the mutated genes may enable the identification of critical drivers involved in liver tumorigenesis or relevant tumor neoantigens.

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Cancer Genome Atlas Research Network. 2017. *Cell.* 169:1327–1341.e23. https://doi.org/10.1016/j.cell.2017.05.046

de Sousa, M., et al. 1994. *Immunol. Lett.* 39:105–111. https://doi.org/10.1016/0165-2478(94)90094-9

El-Serag, H.B. 2011. *N. Engl. J. Med.* 365:1118–1127. https://doi.org/10.1056/NEJMra1001683

Fu, Y., and F.L. Chung. 2018. *Hepatoma Res.* 4:39. https://doi.org/10.20517/2394-5079.2018.29

Moroishi, T., et al. 2011. *Cell Metab.* 14:339–351. https://doi.org/10.1016/j.cmet.2011.07.011

Muto, Y., et al. 2019 *J. Exp. Med.* https://doi.org/10.1084/jem.20180900

Pietrangelo, A. 2010. *Gastroenterology.* 139:393–408: 408.e1–408.e2. https://doi.org/10.1053/j.gastro.2010.06.013

Roessler, S., et al. 2010. *Cancer Res.* 70:10202–10212. https://doi.org/10.1158/0008-5472.CAN-10-2607

Samstein, R.M., et al. 2019. *Nat. Genet.* 51:202–206. https://doi.org/10.1038/s41588-018-0312-8