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doi:10.1038/labinvest.2012.96

Therapeutic resistance from hepatocellular carcinoma stem cells

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Hepatocellular carcinoma (HCC) is an aggressive cancer with a poor response rate. Its incidence is increasing in the United States and Europe. Administration of conventional maximum tolerated dose (MTD) chemotherapy can be compromised by liver dysfunction in HCC patients. Another approach is to use long-term low-dose metronomic (LDM) therapy with conventional chemotherapeutic drugs, which can be less toxic and is known to block angiogenesis as well as having direct toxic effects on cancer cells. Martin-Padura *et al* hypothesized that in addition to minimal toxicity, LDM might target HCC stem cells.

Using an HCC mouse orthotopic xenograft model, the authors found that LDM therapy with cyclophosphamide (LDM-CTX) was much less toxic than MTD with cyclophosphamide. Furthermore, there was a significant survival difference in LDM-CTX mice as compared with the control group. Although LDM-CTX mice exhibited decreased tumor vascularity, examination of their livers revealed nests of nonproliferative cancer cells that were positive for CD13 and interpreted as HCC stem cells, suggesting that LDM-CTX did not kill all HCC stem cells. Further analysis

using a sphere-formation assay indicated that, although LDM-CTX decreased the number of HCC stem cells, a population of CD13 positive cells was particularly resistant to therapy. Cotreatment with a CD13 antagonist (ubenimex) and LDM-CTX demonstrated greater efficacy over LDM-CTX therapy alone. These interesting results highlight the utility of orthotopic mouse xenograft models in the preclinical evaluation of potential cancer therapies. In addition, they demonstrate that dissection of therapeutic responses in carefully controlled studies can inform unique therapeutic strategies that target different populations of cancer cells.

Orai1 in osteoclast and osteoblast differentiation

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Orai1 is a calcium channel known to play an important role in osteoclast development *in vitro*. Normally, depletion of intracellular calcium stores activates STIM1-Orai1-mediated calcium influx. STIM1 is a transmembrane calcium-sensing protein in the endoplasmic reticulum (ER) that undergoes a conformational change

when the ER's calcium is depleted, leading to an interaction with Orai1 that results in cell membrane localization, calcium channel opening, and calcium influx. Inhibition of Orai1 expression inhibits osteoclast formation, which suggests that calcium influx is required for osteoclast formation. This could be regulated by calcium-binding transcription factors such as NFATc1, which is known to play a role in pre-osteoclast fusion.

To study the role of Orai1 *in vivo*, Robinson *et al* used an Orai1^{-/-} knockout mouse. They found that Orai1^{-/-} knockout mice were smaller than heterozygous and wild-type littermates and showed less postnatal growth. While the skeleton was smaller in Orai1^{-/-} knockouts, it developed normally. Further analysis revealed not only a lack of multinucleated osteoclasts but a surprising defect in bone formation. Whereas osteoclasts failed to form, mononuclear cells were capable of minimal bone degradation. In seeking an explanation for the defect in bone formation, the authors found that Orai1 was expressed in human osteoblasts and that pharmacological inhibition of it blocked osteoblast maturation *in vitro*. Overall, these results demonstrate that Orai1 plays roles in both the differentiation and function of osteoclasts and osteoblasts *in vivo*.

Protection against fructose-induced steatosis in mice

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Nonalcoholic fatty liver disease (NAFLD) is associated with obesity and insulin resistance and the development of hepatic steatosis, steatohepatitis, and

even cirrhosis. Recent epidemiological studies have implicated high fructose intake (e.g., in the form of high-fructose corn syrup) in the development of NAFLD. These results have been corroborated in animal experiments using a fructose-rich diet. It has recently been shown that fructose-induced steatosis is associated with activation of Toll-like receptor (TLR)-4-dependent signaling in the liver. There is evidence that metformin (dimethylbiguanide), a drug used in the treatment of type 2 diabetes, may improve liver function in patients with NAFLD.

Using a mouse model, Spruss *et al* tested whether metformin protected against fructose-induced NAFLD. They found decreased levels of the tight junction proteins occludin and ZO-1 in the duodenums of mice on high-fructose diets, along with increased levels of matrix metalloproteinases (MMPs), suggesting that occludin and ZO-1 were degraded by MMPs. There were also increased plasma levels of endotoxin, presumably due to intestinal bacteria. These findings were correlated with activation of TLR-4 signaling by tumor necrosis factor- α , inhibition of plasminogen activator inhibitor-1, and a decrease in cMet-/ApoB-dependent lipid export in the liver. Administration of metformin to mice on high-fructose diets reversed all of these findings; for example, the levels of occludin and ZO-1 in the duodenum increased. Future studies will be required to determine the mechanisms and the overall contribution of intestinal MMP activation to the pathogenesis of fructose-induced NAFLD. From a translational science perspective, these results suggest that metformin may be useful in the prevention of NAFLD.

an ETS protein that targeted SIRT7 to ELK4 binding sites, resulting in repression of a specific gene set relevant to cancer. Depletion of SIRT7 in HT1080 cells inhibited the E1A-dependent reduction of H3K18Ac and escape from contact inhibition. Tumor formation was also impaired in a SIRT7-depleted mouse xenograft model. Together these findings demonstrate that SIRT7 plays a role in cancer maintenance through gene silencing by deacetylation of H3K18Ac.

Nature, published online 6 May 2012; doi:10.1038/nature11043

PREX2 mutations in melanoma Genome-wide sequencing is being used to identify novel mutations in a variety of cancer subtypes. In a recent letter in *Nature*, Berger *et al* described the results of whole-genome sequencing in melanoma. They identified several mutations that had previously been associated with melanoma, including *BRAF*, *NRAS*, and *KIT* mutations. In addition, they identified several novel mutations. Particular attention was paid to *PREX2* (phosphatidylinositol-3,4,5-triphosphate-dependent Rac exchange factor 2), which encodes a protein known to interact with and negatively regulate PTEN. Overall, a 14% frequency of nonsynonymous *PREX2* mutations was identified in a melanoma extension cohort. In addition, ectopic expression of *PREX2* mutants accelerated tumorigenesis in immortalized human melanocytes transplanted into immunocompromised mice. Further work will be required to characterize the molecular mechanism by which *PREX2* mutants drive tumorigenesis.

Nature, published online 9 May 2012; doi:10.1038/nature11071

Dissociation of hepatosteatosis from diabetes in mice Hepatosteatosis, a defining feature of nonalcoholic fatty liver disease (NAFLD), is linked to insulin resistance, but the nature of the relationship is unclear. Histone deacetylase 3 (Hdac3) controls the circadian rhythm of hepatic lipogenesis. During the day, when mice are resting, Hdac3 activity represses the expression of genes involved in lipid synthesis and sequestration, allowing for metabolic precursors to be directed toward gluconeogenesis while Hdac3 is absent, favoring the synthesis of lipids and energy storage. To investigate the relationship between NAFLD and insulin resistance, Sun *et al*, in a study recently reported in *Nature Medicine*, generated hepatocyte-specific *Hdac3*^{-/-} knockout mice. These mice developed severe hepatosteatosis but exhibited insulin hypersensitivity, not insulin resistance, despite normal insulin signaling. The authors were thus able to dissociate NAFLD from insulin resistance, suggesting that, although the two are related, they are not necessarily dependent on each other. These results have profound implications for both the pathogenesis and the treatment of different types of diabetes.

Nature Medicine 2012;18:934–942; doi:10.1038/nm.2744

Mechanism of nicotine-induced abdominal aortic aneurysm Smoking, which increases the risk of abdominal aortic aneurysm (AAA) 7.6-fold, is the only modifiable risk factor associated with AAA. However, the mechanistic relationship between nicotine and AAA is unclear. Recently, several pieces of evidence suggested that AMP-activated kinase (AMPK) might be involved in AAA formation. Wang *et al* investigated the relationship between smoking, AMPK, and AAA formation and reported their findings in *Nature Medicine*. They found that nicotine leads to development of reactive oxygen species, which activates aortic smooth muscle AMPK, which phosphorylates the transcription factor activator protein 2 α , which leads to the transcription of matrix metalloproteinase 2, which is directly responsible for extracellular matrix degradation and AAA formation.

Nature Medicine 2012;18:902–910; doi:10.1038/nm.2711