

Investigating the Genetic Mechanisms Underlying Cholangiocarcinoma

What role do key mutations play in causing this lethal liver cancer?

Intrahepatic cholangiocarcinoma (ICC) is a type of liver cancer with a poor prognosis and rising incidence. Three years ago, Massachusetts General Hospital researchers found mutations in genes encoding the enzymes IDH1 and IDH2 in about 25 percent of cases of ICC. These mutations are also seen in brain tumors and in certain types of leukemia and bone cancer.

The mutant forms of IDH1 and IDH2 are thought to cause cancer through an unusual mechanism: by producing, at very high levels, a rare metabolite, 2-hydroxyglutarate (2HG), which is not normally present in the cell. Non-mutated IDH produces

α -ketoglutarate (α KG), a molecule that is used to generate energy and to produce fats and other building blocks of the cell as well as serving as a co-factor used by many other enzymes. Mutant IDH, on the other hand, converts α KG to 2HG, which interferes with enzymes that require α KG.

However, the mechanisms by which mutated IDH led to tumor formation were still unclear. So the researchers developed a genetically engineered mouse model of IDH and used it to discern the mechanism of action of mutated IDH. Their study, published in the September 2014 issue of *Nature*,¹ showed that the IDH mutation prevents cellular differentiation and creates a vulnerable cell state primed for transformation by other oncogenes.

Their work also created a viable mouse model system for continued study of ICC with mutated IDH.²

A MUTATION THAT BLOCKS HEPATOCYTE DIFFERENTIATION

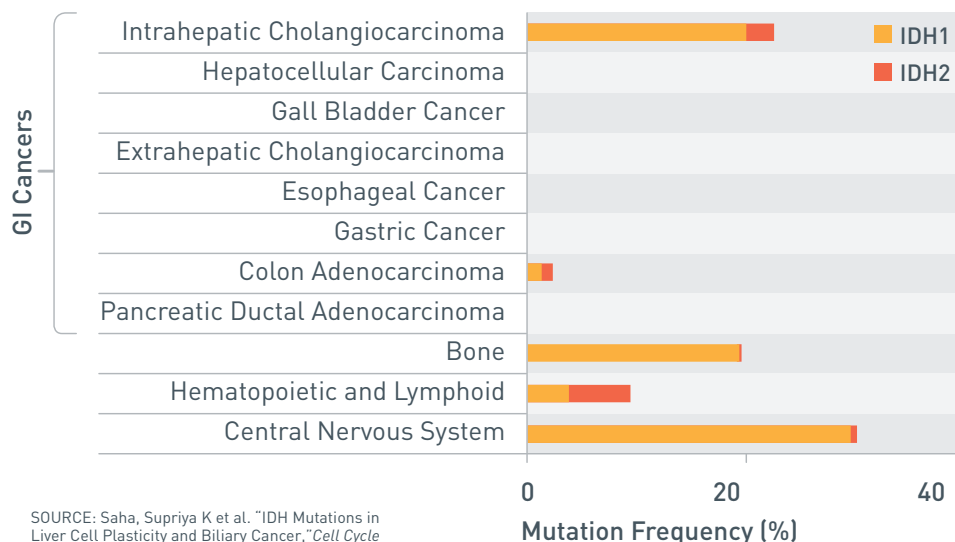
Supriya Saha, MD, PhD, Christine Parachoniak, PhD, and Nabeel Bardeesy, PhD, researchers at the Mass General Cancer Center's Center for Cancer Research, led these studies with the help of a team of collaborating scientists. They hypothesized that mutated IDH might block liver cells in an immature or undifferentiated state. To address this, they first employed an *in vitro* model system of liver cell differentiation using a type of liver stem cell called a hepatoblast.

The team found that mutated IDH completely blocked the ability of stem cells to mature into differentiated hepatocytes. They discovered that the mutation did so by suppressing the production of HNF-4 α , a master regulator of hepatocyte differentiation. To prove that 2HG is the mechanism that blocks differentiation, the researchers added 2HG to a normal, non-mutated stem cell, and indeed, differentiation was blocked. By contrast, a drug that inhibited mutant IDH from producing 2HG completely restored the ability of cells with this mutation to become hepatocytes.

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IDH1 & IDH2 Mutations and Cancer Incidence

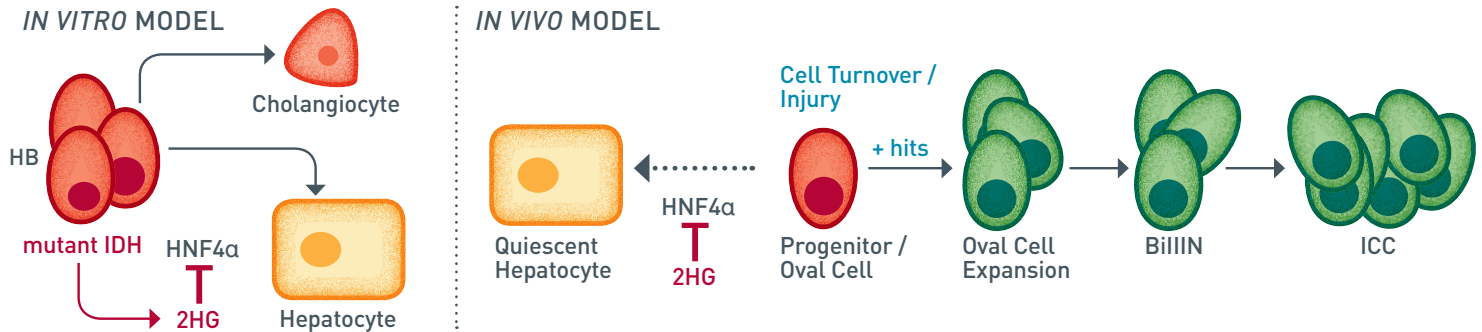
This chart displays the frequency of each tumor type exhibiting a mutation in either IDH1 or IDH2, present in more than 20 percent of ICC but rare or absent in other GI cancers, as well as the frequency of IDH mutations in other tissues, such as the CNS and bone.



SOURCE: Saha, Supriya K et al. "IDH Mutations in Liver Cell Plasticity and Biliary Cancer," *Cell Cycle* 13, no. 20 (October 2014), 3176-82.

Proposed Model of IDH Mutations

The left panel shows expression of mutant IDH in a liver hepatoblast (HB) leading to production of 2HG, blocking hepatocyte differentiation through suppression of HNF4 α . On the right, IDH acts in the adult liver to block oval cell differentiation. These cells are sensitized to transformation by additional oncogenic hits, and can progress through graded premalignant biliary lesions, ultimately leading to ICC.



SOURCE: Saha, Supriya K et al. "IDH Mutations in Liver Cell Plasticity and Biliary Cancer," *Cell Cycle* 13, no. 20 (October 2014), 3176-82.

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CREATING A MOUSE MODEL TO TEST THE IMPACT OF MUTATED IDH

To further understand the process by which mutated IDH leads to ICC, the team developed a mouse genetically engineered to express mutated IDH in the liver. Yet while this produced large amounts of 2HG, it had no effect on the biology of the healthy adult liver. The team concluded that this was because the liver consists of fully mature cells unless the liver is injured, so they altered the mouse model by feeding it a chemical that would slightly injure hepatocytes. Based on prior work, this chemical was known to activate an oval cell, a type of undifferentiated cell resembling the

embryonic hepatoblasts used in the *in vitro* model.

In the control mice, the liver was slightly damaged by the introduction of the chemical, but recovered and returned to normal after one month. Notably, in the livers of mice with mutated IDH, the HNF-4 α gene was turned off and the oval cells continued to divide, losing their ability to differentiate into mature liver cells.

COMBINED EFFECT OF MUTANT IDH AND KRAS ACCELERATE CELL DIVISION

Still, this injury alone did not lead to development of tumors. The researchers hypothesized that while mutant IDH alone is not sufficient to cause cancer, its ability to block

liver cells from differentiating may result in a state vulnerable to the impact of additional oncogenic mutations. In this regard, like IDH, KRAS is often mutated in human ICC tumors, but genetically engineered mice with KRAS oncogene by itself are not highly prone to ICC. Next the research team bred the mutant IDH mouse with the KRAS model. In the offspring, they found that the combined effect of mutant IDH and KRAS was accelerated division of the oval progenitor cells and the rapid development of ICC that closely modeled the multistage tumor progression that characterizes the human disease.

A NEW MODEL FOR ICC

The team is exploring the potential of using the newly developed mouse model to study the effects of drugs that can block mutated IDH, which they believe may restore the ability of the tumor cells to differentiate and therefore lose their malignant properties. The results of such studies will be vital to finding a treatment for ICC caused by this mutation. ■

¹Saha, Supriya K, Christine A Ghanta, Krishna S Fitamant, Ross Julien, Kenneth N, et al. "Mutant IDH Inhibits HNF-4 α to Block Hepatocyte Differentiation and Promote Biliary Cancer," *Nature* 513, no. 7516 (September 2014), 110-14.

²Saha, Supriya K, Christine A Parachoniak, Nabeel Bardeesy. "IDH Mutations in Liver Cell Plasticity and Biliary Cancer," *Cell Cycle*, published online September 2014. <https://www.landesbioscience.com/journals/cc/article/965054/?nocache=802180651>.

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