# Research isolates liver cancer stem cells prior to tumor formation

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Penn State College of Medicine researchers, in collaboration with colleagues at the University of Southern California, have taken an important step in understanding the role of stem cells in development of liver cancer. Using a unique approach that involves study of individual cells, the team, led by C. Bart Rountree, M.D., has demonstrated for the first time a population of cancer stem cells in the liver prior to tumor formation. The research, published in the journal *Stem Cells,* shows a potential link between liver stem cells and liver cancer.

Using a liver-specific PTEN (phosphatase and tensin homolog deleted on chromosome 10) mouse model allowed Rountree and his colleagues to study the microenvironment of the liver without affecting the rest of the mouse.

"The PTEN knock-out mouse is one model of chronic liver injury that ultimately leads to liver cancer. During chronic injury, liver stem cells proliferate, and at times of healthy liver, the liver stem cells are very rare," Rountree said. "We were initially looking for what is driving liver stem cell proliferation during chronic liver injury.

"We started investigating liver stem cells in many different liver injury models with the idea we may be able to help people with liver disease, but we discovered that some cells we isolated were malignant," Rountree said. "It was quite a surprise for us because there were not any tumors in the mice when we isolated the cells."

The liver is the only organ in the body that is able to fully regenerate itself. The cells of the liver, including hepatocytes and cholangiocytes, can divide and repopulate themselves. With chronic liver injury, including by a virus or alcoholism, the hepatocytes lose the ability to make more of themselves. In that setting, liver stem cells proliferate and can make either of the cell types. However, patients with chronic injury also develop liver cancer, opening the possibility that the stem cells are involved in tumor formation.

"There's been a groundswell of interest in understanding the role of specific stem cells in the development of liver cancer," Rountree said. "There is a cancer stem cell lurking out there that may be very bad. It has stem cell properties and is malignant, resistant to chemotherapy. These properties make it harder to treat these cancers.

"What we ended up doing was shifting our understanding of liver stem cells and their role in malignancy," Rountree said. "All work previously done was looking at patients, animal models or cell lines after the tumor already developed. What we did was identify malignant stem cells before there is evidence of the primary tumor. This gave us a new perspective on not only what the potential of stem cells for therapy is, but also in terms of what's driving cancer formation. Imagine treating a cancer before a primary malignancy forms."

Researchers created ten cell lines to study using a single-cell isolation technique. Cells that make a unique surface protein called CD133 were separated by placing them in a liquid medium and running through a flow cytometer. Once identified, a robot took a single CD133-positive cell and placed it in a single drop into one well of a culture dish. Doing this several hundred times, the cell lines were established.

These single cells, when expanded up, had stem cell characteristics, having markers of both hepatocytes and cholangiocytes. When these

lines were injected into a mouse with a deficient immune system, the tumors then formed.

Rountree said there is interest in targeting these stem cells with malignant potential. "Can we target these cells in patients with hepatitis B or C, either before or after their cancer forms?" Rountree said. "The broader implication is very powerful. If you look at a patient with chronic injury and find a way to specifically target cells with malignant potential, you may be able to prevent liver cancer in the first place."

Source: Pennsylvania State University (news : web)