MedicalXpress

After three decades of searching, scientists find cellular targets of Hepatitis B virus

October 22 2012

A University of Colorado Boulder-led team has discovered two prime targets of the Hepatitis B virus in liver cells, findings that could lead to treatment of liver disease in some of the 400 million people worldwide currently infected with the virus.

CU-Boulder Professor Ding Xue, who led the studies, said scientists have been looking for cellular targets of the Hepatitis B virus, or HBV, for more than three decades. Infections from HBV promote hepatitis (inflammation of the liver), cirrhosis (scarring of the liver) and liver cancer and can be transmitted through blood and bodily fluids, unprotected sex, unsterile needles and from infected mother to offspring during birth.

Xue said scientists have known for some time that HBV encodes a pathogenic, tumor-promoting protein known as HBx, but how it works has remained largely unknown. In two new studies, Xue and his colleagues showed that the "host targets" of HBx in human cells are two small cell proteins known as Bcl-2 and Bcl-xL, both of which are well-known cell death inhibitors but which have not previously been implicated in HBV infection.

HBx uses a particular "motif," a small string of protein building blocks known as amino acids that resemble those seen in some cell death-causing proteins, to interact with the Bcl-2 and Bcl-xL targets and stimulate an elevation of calcium in the host cell. The calcium elevation then triggers both viral HBV replication and cell death, said Xue.

When the researchers introduced gene mutations into the motif, HBx binding to the Bcl-2 and Bcl-xL proteins and viral replication were prevented. Similarly, when either Bcl-2 or Bcl-xL proteins were "knocked out" or weakened in human liver cells, HBx was less able to cause an increase in calcium and viral replication inside the infected cells.

"Our most important findings are the identification of the motif itself and the two HBx host targets," said Xue of CU-Boulder's molecular, cellular and developmental biology department. "Now we can start thinking about new drug targets to treat HBV."

Two papers on the subject led by Xue were published online Oct. 22 in the *Proceedings of the National Academy of Sciences*. In addition to major CU-Boulder co-authors Xin Geng, Brian Harry, Qinghua Zhou, Yan Qin and Amy Palmer, a group led by Professor Ning-Shao Xia from the National Institute of Diagnostics and Vaccine Development in Infectious Diseases at Xiamen University in China collaborated on one of the studies.

The World Health Organization estimated in July that about 600,000 people die annually from acute or chronic HBV infection, which is most predominant in Asia and Africa.

In one of the *PNAS* studies, the authors used a tiny roundworm known as C. elegans, a widely used animal model in biomedical research, to identify HBx host targets within the cell. Xue and his team showed that HBx can induce cell death in C. elegans through a protein known as CED-9, mimicking an early stage of liver infection by HBV.

Previous work had shown CED-9 in C. elegans is a homolog of the human Bcl-2 protein—a different protein in a different animal that has a similar function. Despite the stark differences between roundworms and

humans, scientists estimate 35 percent of C. elegans genes have human homologs.

"Our results suggest that C. elegans can serve as a good animal model for identifying crucial host factors and cell signaling pathways and aid in development of strategies to treat HBV-induced liver disorders," said Xue. "I think the use of C. elegans will galvanize the field of HBV study, which has been in search of a good animal model for three decades."

Simple animal models like fruit flies and roundworms have been critical for understanding fundamental biological processes such as aging, cell death and the regulation of gene expression. "Many would not have considered using C. elegans as a model to study HBV, but the genetic 'tools' of C. elegans are ideal for the identification of viral host targets, even though C elegans is not a native host for the virus," said CU's Harry. Harry is pursuing both a Ph.D. degree in MCD Biology at CU-Boulder and an M.D. at the CU School of Medicine on the Anschutz Medical Campus in Aurora through the Medical Scientist Training Program.

"Both studies show that if you create two mutations in this small HBx motif, it takes away its ability to bind to Bcl-2 family proteins. This wipes out viral replication and host cell death caused by HBx expression," said Harry.

Xue said there currently is no effective treatment for chronic HBV carriers, although some people with chronic HBV are treated with interferon and anti-viral drugs. But such treatments are either unavailable or too expensive in developing countries where most of the HBV infections are occurring. "That's why these new findings could have profound clinical and pharmaceutical implications for the treatment of HBV patients," he said.

Harry said the Hepatitis B vaccine, which was developed in 1982, is administered around the world and has been shown to work well in preventing new infections. "The problem is that once you are infected, there is no effective way to remove the virus from the body," he said. "When the virus replicates in liver cells, it causes cycles of cellular damage, inflammation and tissue regeneration, resulting in the accumulation of genetic mutations and liver cancer."

HBV is 50 to 100 times more infectious than the HIV virus, according to WHO officials. In China and other parts of Asia, most people acquire HBV during childhood and 8 to 10 percent of the adult population is chronically infected. "Because of this, understanding how HBV and HBx cause pathogenesis can have dramatic clinical impact," said Xue.

Provided by University of Colorado at Boulder