Previously unknown hepatitis B virus pathway from the cell identified

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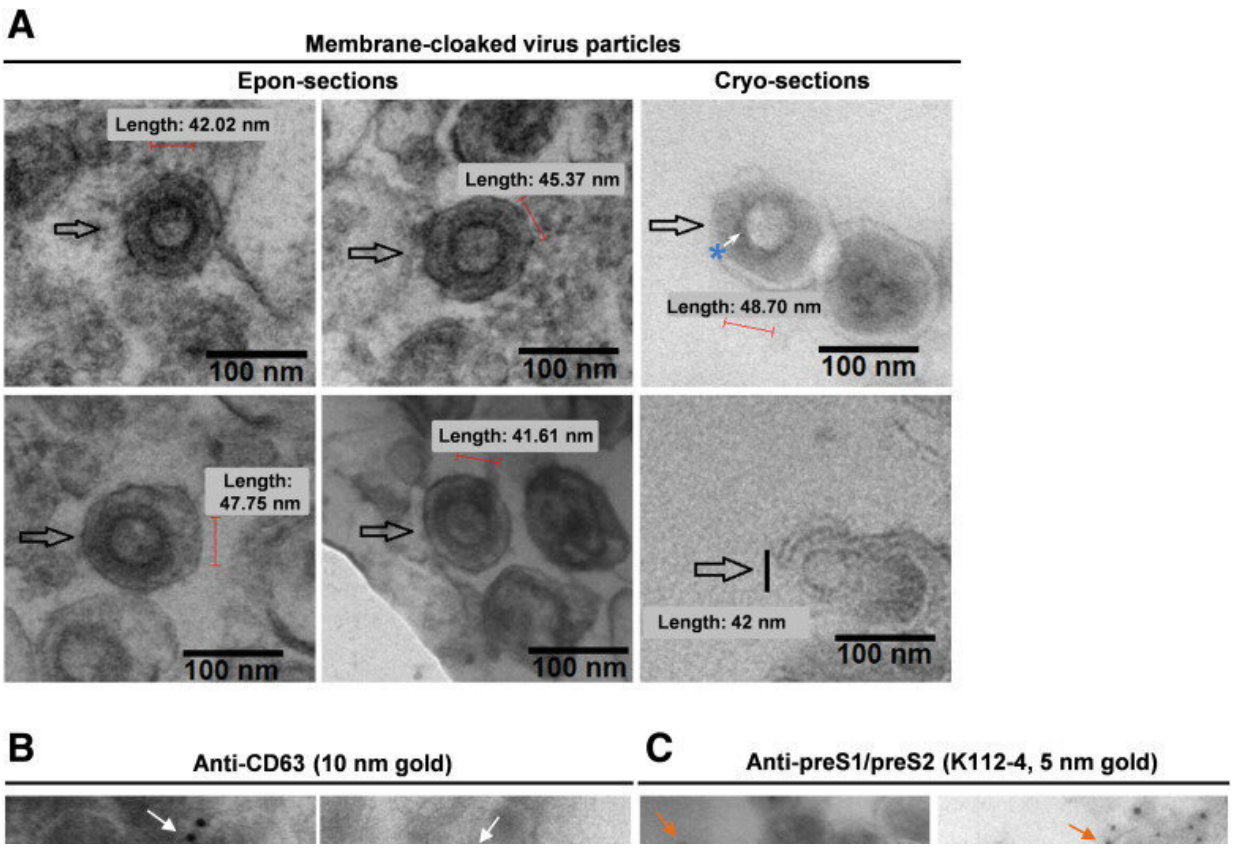

Transmission electron microscopy of exosomes released from HepAD38 cells. A, TEM images from ultra-thin sections of Epon-embedded (left) or cryo-sections (right) of fixed exosomes showing virions enclosed by a membrane structure (labeled by arrows). An asterisk indicates that a dense viral envelope stands out from the surrounded nucleocapsid. B–C, Immunogold labeling of ultra-thin thawed cryo-sections of fixed exosomes. The cryo-sections were either labeled with an anti-CD63 antibody (visualized by 10-nm gold particles) or an anti LHBs antiserum (anti-preS1/preS2 domain rabbit serum [K112-4])

(visualized by 5-nm gold particles). Arrows indicate specific colloidal gold labeling. Asterisks represent that anti-LHBs (5 nm) is located at identifiable enclosed virus like particles. The surface of cryo-sectioned exosomes was also labeled by anti-LHBs (orange arrows). Credit: *Cellular and Molecular Gastroenterology and Hepatology* (2022). DOI: 10.1016/j.jcmgh.2022.09.012

Around 1.5 million people worldwide become infected with the hepatitis B virus (HBV) every year. A research team led by the Paul-Ehrlich-Institut has identified a previously unknown pathway for the egress of hepatitis B virus particles from cells: researchers were able to visualize intact virus particles in extracellular vesicles (exosomes) for the first time.

Exosomes, small vesicles in cells that are released into the surroundings, serve as a means of transport and possibly as a protective covering. *Cellular and Molecular Gastroenterology and Hepatology* reports on the results in its online edition from September 29, 2022.

Hepatitis B is one of the most common infectious diseases in history. It is caused by the hepatitis B virus (HBV), which can cause acute and chronic hepatitis. An estimated 300 million people worldwide suffer from chronic hepatitis B infection, which is associated with an increased risk of developing cirrhosis of the liver or liver cell carcinoma.

Every year, around 900,000 people worldwide die as a result of an HBV infection. Serological evidence even shows that an estimated two billion people have previously been infected or are currently infected with HBV. HBV is transmitted mainly via blood, but also via other body fluids such as saliva, lacrimal fluid, sperm or even vaginal secretions.

Although safe and effective vaccines have been authorized, HBV

continues to pose a major health problem. Many countries under a high epidemic burden do not have effective vaccination strategies or lack vaccine supplies. It is also a medical challenge to completely cure a chronic infection.

New hepatitis B virus egress pathway identified

Much is now known about the life cycle of HBV. Nevertheless, there are still parts that have not been fully elucidated, such as which mechanisms enable the virus to leave the cell. Like all viruses, HBV needs to be released from the cell to reproduce and to infect other cells. Various studies have shown that some viruses can be found in exosomes. These small vesicles are released into the surroundings by the cell. They are used for cellular communication and the transport of molecules.

A research team from the Paul-Ehrlich-Institut, headed by Professor Eberhard Hildt, head of the Virology Division, investigated whether hepatitis B viruses also use exosomes as a means of transport from the cells. It was not yet known whether this was a mechanism used by HBV.

In their work, the research group isolated exosomes from the cell culture supernatant of HBV-producing human liver cells and found both typical exosomal markers and markers of hepatitis B viruses. These exosomal fractions were separated from fractions containing free virions to determine whether hepatitis B viruses were actually present in the exosomes. Virions are virus particles that are located outside of cells.

It was possible for the research team to release intact HBV virions stepwise from the exosomes with the help of detergents, which dissolve the membrane of the exosomes.

The scientists were able to observe that inhibiting exosome morphogenesis in the laboratory impaired the release of exosome-

wrapped HBV. In addition, electron microscopy confirmed the presence of intact virions in the examined exosomes. The research team also detected the presence of the HBV surface protein LHB (large hepatitis B virus surface antigen) on the surface of exosomes.

The presence of this protein makes it possible for the hepatitis B viruses encapsulated in exosomes to infect susceptible cells. An uptake of exosomal hepatitis B viruses was also observed in cells which are usually not very susceptible to HBV infection, albeit with low efficiency.

The current research data indicates that some of the intact HBV virions can be released as exosomes. This is a release pathway for hepatitis B viruses which had not been previously identified. Although hepatitis B, C and E viruses are completely different, they all use this pathway to leave cells.

The research was published in *Cellular and Molecular Gastroenterology and Hepatology*.

More information: Qingyan Wu et al, Presence of Intact Hepatitis B Virions in Exosomes, *Cellular and Molecular Gastroenterology and Hepatology* (2022). DOI: 10.1016/j.jcmgh.2022.09.012

Provided by Paul-Ehrlich-Institut