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https://www.gesundheitsindustrie-bw.de/en/article/news/alternativer-infektionsweg-fuer-grippeviren-durch-neues-testverfahren-entdeckt

New test assay leads to discovery of new influenza virus infection route

Researchers from the University of Freiburg have recently discovered a completely new mechanism that influenza viruses use to infect cells. This discovery was partly made possible by a so-called emulsion coupling assay - an extremely sensitive, digital detection method developed by Actome GmbH in collaboration with scientists from the Freiburg University of Applied Sciences and Hahn-Schickard. The assay is used to count individual molecules and investigate how they interact.

Influenza infection is caused by the influenza virus entering the body through the mucous membranes and proliferating in the respiratory tract. The pathogen docks to the host cell and passes through the cell membrane into the cell interior. While it was previously thought that the interaction between virus and cell only occurred with the help of sugar molecules, it has now been demonstrated that proteins can play the same role as sugar molecules. This discovery is a world first.

A team of researchers from the Institute of Virology at the Freiburg University Medical Centre, the Chair of Application Development in Microsystems Technology at the Department of Microsystems Engineering (IMTEK) and Hahn-Schickard have recently demonstrated a completely new mechanism that influenza viruses use to enter cells. The researchers found that bat influenza viruses use a completely different route of entry into the host cell than all previously known influenza viruses. Bat influenza viruses infect animal and human cells by utilising MHC (major histocompatibility complex) class II proteins rather than binding sialic acid moieties on the host cell surface. MHC class II molecules play a major role in the functioning of the immune system and are very similar in all vertebrates. This is what makes the researchers' discovery so important: as mice, pigs, chickens and humans have virtually the same entry portals for the virus, it is conceivable that bat influenza viruses are transmitted naturally from bats to other vertebrates, including humans.

Sensitive detection method detects individual molecules

The virologist Dr. Csaba Jeney has developed the emulsion coupling assay, a sensitive method for studying the interaction of protein molecules.

A new test procedure called emulsion coupling assay, developed by Actome GmbH, an IMTEK spin-off, was used to unambiguously confirm that the new mechanism works. Actome GmbH's assay provides a highly sensitive analysis of the interaction between protein molecules. "The problem with influenza viruses is that virus-cell interaction takes place at such a low level that we have not been able to identify these interactions using traditional methods because they are not sensitive enough," explains the CEO of Actome, Dr. Csaba Jeney, who developed the assay with his research group at IMTEK. "We use two antibodies for detection: one for the virus, and another for the host cell. These two antibodies are combined into a complex, enclosed in a single drop of liquid and then further diluted. This produces many thousands of tiny microdroplets, each of which contains a protein molecule. Another very important feature of our concept is that no further washing step is required. Further washing steps run the risk of losing the tiny number of molecules that we want to detect."

Thousands of reaction chambers consisting of fluid droplets

The droplets with the encapsulated molecules can be produced using an analysis method that uses microfluidic technologies in an oil environment. The Freiburg researchers' invention enables the formation of around 20,000 different reaction chambers in a water-in-oil emulsion, with a single detection reaction subsequently taking place in each chamber. "In the case of influenza viruses, we were looking for droplets that showed positive for both antibodies," says Jeney. "This showed us that the virus had bound to the MHC membrane proteins. If the virus did not bind to the MHC membrane proteins, the two antibodies were found in different droplets." The researchers were able to successfully demonstrate that the bat influenza virus bound to proteins of the MHC II complex. "In contrast to traditional methods such as immunoprecipitation or ligation, we now have a highly sensitive tool that enables us to study protein-protein interactions," says Jeney.

For safety reasons, only recombinant influenza viruses and test cells that are not viable on their own were used for the experiment, which provided the researchers with a defined system. The researchers initially tried to remove the viruses from the cells by washing. Connections were fixed with chemicals, the cells lysed and the two antibodies added to find out whether there was any interaction between the cells.

Method enables absolute quantification of protein molecules

Emulsion coupling assay schematic: the highly sensitive detection method involves the creation of fluid droplets containing antibodies of proteins of interest. Thousands of small reaction chambers are created in an oil environment where both PCR and the subsequent detection reaction occur.

Various methods, including methods used for producing droplets used for digital PCR, can be used for preparing the droplets for the emulsion coupling assay. "We have our own emulsification process facilities at IMTEK, which we lent to the virologists for their experiments," says Jeney. The reaction droplets are collected, amplified by PCR and individually analysed using a flow cytometer. Each droplet has a certain fluorescence value, which can be used to determine whether the virus has bound or not. The ratio of positive to negative signals is then mathematically calculated and quantified absolutely. "And that's exactly what makes our method so powerful," says Jeney. "As every droplet contains exactly one molecule, we can count how many protein-protein complexes were actually formed." The researchers want to use this in the future to gain insights into the development of other diseases such as cancer. The emulsion coupling assay enables us to study virtually all protein-protein interactions in the cell," says Jeney. "And it is very simple to achieve. We can then use the assay to find out what is happening in the cells when something goes wrong."

Start-up wants to decode proteins for use in personalised therapies

The scientists have filed a patent for the new method. They want to commercialise it in partnership with Actome GmbH, the IMTEK spin-off. "The plan is that Actome will bring the assay to market," explains the scientist. "Our vision is to work with Actome to decode signal transduction pathways, their proteins and their interaction in the body. Admittedly, this would involve a lot of basic research, but our work would also provide important information needed by the pharmaceutical industry to develop new drugs and diagnostic procedures." In addition, Jeney and his team plan to develop different versions of the assay over the next few months so that they can directly examine single cells.

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The article is part of the following dossiers

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