Molecular imaging may lead to earlier diagnosis of childhood respiratory virus

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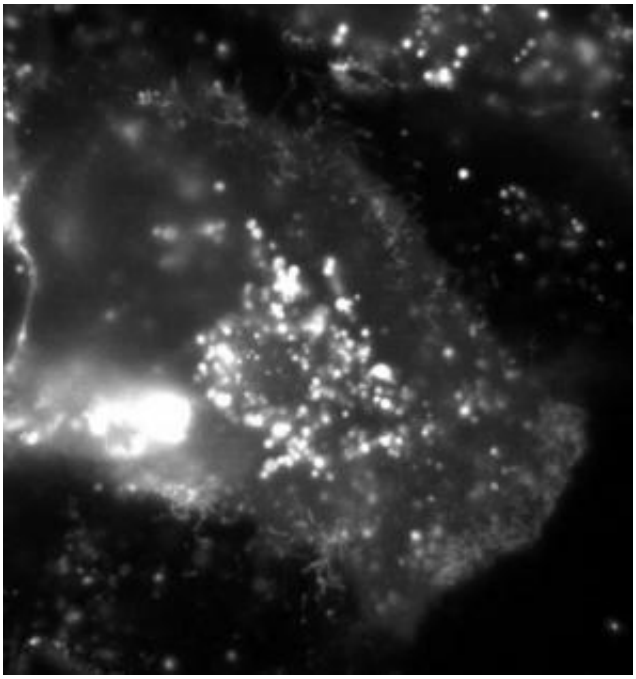

An epi-fluorescence microscopy image shows human RSV viral RNA in aggregates, called inclusion bodies, and in filament form, growing in green monkey kidney cells, using molecular-scale probes called molecular beacons molecular beacons. Credit: Image Courtesy of Phil Santangelo

Scientists have used a powerful molecular imaging technique to see inside living cells infected with the most pervasive and potentially fatal childhood respiratory virus known to medicine -- respiratory syncytial virus (RSV). The technique is yielding insight on viruses – such as RSV,

human influenza, hepatitis C, West Nile virus and severe acute respiratory syndrome (SARS) -- that replicate with the help of proteins encoded by ribonucleic acid (RNA) inside the cell. Ultimately, the research could to lead to early and rapid detection of viral infection and the design of new antiviral drugs.

Scientists and engineers at the Georgia Institute of Technology and the University of Georgia are studying bovine and human RSV with molecular-scale probes – called molecular beacons – that are engineered oligonucleotides (short sequences of RNA or DNA) shaped like a hairpin with a fluorescent dye molecule on one end and a quencher molecule on the other end. They are designed to fluoresce only when they bind to a complementary target – in this case, RSV genomic RNA.

"For the first time, we were able to visualize an important part of the RSV virus -- its genome -- in live, infected cells," said Phil Santangelo, a research engineer in the Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University. "Our molecular beacons attach to the virus and glow inside infected cells as the virus grows, replicates and infects other cells. We can now see that happen in real time in cultures in the lab.

"That's very different from how scientists have studied viruses in past; they've looked at viruses in fixed (or preserved) cells," he added. "…. Within the first week of studying human RSV in living cells, I learned something new because I was looking at it live."

Molecular beacons were originally developed at the Public Health Research Institute in New Jersey in the late 1990s. They were initially used for in vitro assays outside cells. But Santangelo and former Georgia Tech Ph.D. student Nitin Nitin, now a postdoctoral researcher at Rice University, devised methods for getting the beacons inside the cell without destroying the probe and without changing the cells.

Santangelo will give an invited presentation on his research on April 20 at the Materials Research Society meeting in San Francisco. The research is funded under a National Institutes of Health grant to Professors Shuming Nie and Gang Bao – both in the Department of Biomedical Engineering at Georgia Tech and Emory -- to develop new, high-sensitivity live-cell probes. In this study, Santangelo, who works for Bao, collaborated with Amelia Woolums, an associate professor of large animal medicine at UGA.

They determined their molecular beacon techniques deliver high-sensitivity and high-specificity results in both bovine and human RSV strains. "The RSV genome is interesting in that it is 15,000 nucleotides long, and one of its RNA sequences repeats itself nine times," Santangelo explained. "So we were able to bind up to nine probes to that sequence, and that helped us achieve very high sensitivity to the virus. In the human virus, in fact, we were able to see a single RSV virion."

Also, researchers were able to detect virion aggregates in bovine RSV within the first day in culture, Santangelo noted. Typically, veterinarians cannot detect RSV until after five or six days of incubation.

Bovine RSV can be a major problem in cows, which represent a good animal model for human RSV. Calves have RSV symptoms similar to those in human babies, and the disease pathology is similar. So studying bovine RSV yields information about the strain that infects humans, he added. Also in this study, researchers used confocal microscopy to view very thin sections of the RSV viral genome in live, infected cells. This technique allowed them to reconstruct the viral RNA aggregates in three dimensions.

"Most pathologists look at thick sections of RSV in formaldehyde, but our 3D structures are more fluid and amorphous than the solid structures pathologists have observed," Santangelo said. "The more we know about

how RSV really looks, the more we'll understand about its pathogenesis."

RSV is the most important cause of respiratory infection in young children worldwide, infecting virtually every child in the first few years of life. Immunity is feeble and fleeting, and repeated infections are the rule. One in every 100 or 200 infected infants requires hospitalization, usually for bronchiolitis. There is not yet an effective vaccine for RSV, and current anti-viral drugs are in their infancy in terms of efficacy, Santangelo noted.

Ultimately, researchers want to conduct in vivo testing, but must first adapt their molecular beacons technology for that purpose, Santangelo said. "In the nearer term, we hope to use molecular beacons to detect RSV in clinical samples like with those taken with a nasal swab. We might be able to detect RSV in its first day of incubation and make an early diagnosis," he added.

The researchers also hope their research will lead to development of a suite of anti-viral drugs for treating RSV and other viruses, including human influenza.

Source: Georgia Institute of Technology