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14-18 minutes

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COVID-19 News: A new study by researchers from Whitehead Institute for Biomedical Research-Cambridge, Wyss Institute for Biologically Inspired Engineering-Harvard University, Department of Biology-Massachusetts Institute of Technology and the John A. Paulson School of Engineering and Applied Sciences-Harvard University have alarmingly discovered that the SARS-CoV-2 RNA is reverse-transcribed and integrated into the human genome. These study findings have a wide range of implications and could change the course of medical science and human genetics along with a wide range of other unimaginable implications.

The study findings were published on a preprint server and are currently being peer reviewed while also causing a phenomenal stir within the medical and research community. https://www.biorxiv.org/content/10.1101/2020.12.12.422516v1

Individuals who recover from COVID-19 sometimes later test positive for SARS-CoV-2, suggesting their immune systems could not ward off a second attack by the coronavirus or that they have a lingering infection. This now hints at a different explanation in which the virus hides in an unexpected place. The study suggests the pandemic pathogen takes a page from HIV and other retroviruses and integrates its genetic code but, importantly, just parts of it into human's chromosomes. The phenomenon, if true and frequent, could have profound implications that range from false signals of active infection to misleading results from COVID-19 treatment studies.

Prolonged SARS-CoV-2 RNA shedding and recurrence of PCR-positive tests have been widely reported in patients after recovery, yet these patients most commonly are non-infectious.

In this stud the researchers investigated the possibility that SARS-CoV-2 RNAs were being reverse-transcribed and integrated into the human genome and that transcription of the integrated

sequences might account for PCR-positive tests.

In support of this hypothesis, the study team found chimeric transcripts consisting of viral fused to cellular sequences in published data sets of SARS-CoV-2 infected cultured cells and primary cells of patients, consistent with the transcription of viral sequences integrated into the genome.

To experimentally corroborate the possibility of viral retro integration, the study team describe evidence that SARS-CoV-2 RNAs can be reverse transcribed in human cells by reverse transcriptase (RT) from LINE-1 elements or by HIV-1 RT, and that these DNA sequences can be integrated into the cell genome and subsequently be transcribed.

Human endogenous LINE-1 expression was induced upon SARS-CoV-2 infection or by cytokine exposure in cultured cells, suggesting a molecular mechanism for SARS-CoV-2 retro-integration in patients. This novel feature of SARS-CoV-2 infection may explain why patients can continue to produce viral RNA after recovery and suggests a new aspect of RNA virus replication.

Typically all viruses insert their genetic material into the cells they infect, but it generally remains separate from the cell's own DNA.

Molecular biologist Dr Rudolf Jaenisch of the Massachusetts Institute of Technology (MIT), who led the work was intrigued by reports of individual testing positive for SARS-CoV- 2 after recovering, wondered whether these puzzling results reflected something of an artifact from the polymerase chain reaction (PCR) assay, which detects specific virus sequences in biological samples such as nasal swabs, even if they are fragmented and can't produce new viruses. "Why do we have this positivity, which

is now seen all over the place, long after the active infection has disappeared?" says Dr Jaenisch, who collaborated with the lab of MIT's Richard Young.

Reverse transcriptase (RT) activity has been detected within human cells, as well as integration of the reverse transcription products. For instance, with *APP* gene transcripts, their integration into neuronal genomes by endogenous RT was followed by APP transcription. https://pubmed.ncbi.nlm.nih.gov/30464338/

Typically such endogenous RT is potentially present in the form of human LINE-1 elements, which make up 17% of the human genome. These are autonomous retrotransposon elements that can transpose themselves as well as other elements of the genome back into the DNA of the nucleus for future transcription. https://pubmed.ncbi.nlm.nih.gov/28745987/

https://pubmed.ncbi.nlm.nih.gov/12682288/

https://pubmed.ncbi.nlm.nih.gov/29596909/

Findings Supported By Chimeric Transcripts Present In Published RNA Sequences

The study team looked at the published RNA-sequences from SARS-CoV-2 infected cells. Their aim was to find chimeric transcripts, melding human and viral RNA into the same genome. They found a good number of such reads in several different cell types, from the heart, brain, lung and stomach and from cells retrieved from the bronchoalveolar lavage fluid (BALF) obtained from COVID-19 patients.

Interestingly the proportion of such chimeric sequences was directly correlated with the level of viral RNA in each sample, and

such reads typically made up between 0.004% - 0.14% of the total viral reads. The greatest proportion was in BALF cells from severe COVID-19 patients, at ~69%. There were almost none in blood cells, on the other hand.

Also of the host-viral chimeric protein contained the nucleocapsid (N) sequences, as expected since this is the most abundant viral subgenomic RNA. This would, therefore, be the most likely to be reverse transcribed and then integrated. These findings support the occurrence of this event within infected cells.

Determination Of Sources of RT Activity

The study team conducted an experiment inducing the overexpression of human LINE-1 elements or HIV-1 RT, in the cell line. The three types of RT examined include: LINE-1 overexpression driven by a CMV promoter, LINE-1 overexpression driven by 5' UTR, which is its natural promoter, and HIV-1 RT expression.

The cells were then infected with SARS-CoV-2. At two days post-infection, they carried out polymerase chain reaction (PCR) tests to detect the viral sequences, using the N-targeting primer sets used in the commonly used COVID-19 PCR tests.

Subsequent PCR amplification of the purified cell DNA from infected cells showed the presence of the N protein bands.

This however did not occur in non-transfected or uninfected cells.

The study team next purified the cellular genomic DNA (gDNA) from cells that overexpressed RT and carried out quantitative PCR (qPCR) to confirm the presence of the N sequences.

Significantly, overexpression of CMV-LINE-1 led to an 8-fold rise

in N-sequence signal strength. This indicated a higher number of N sequences were integrated into the genome in these cells, relative to 5' UTR-LINE-1 expression or HIV-1 RT expression. They were able to clone the full-length N DNA from cells overexpressing the first RT type, but not the other two. This might be because of the lower number of N sequences integrated into the host genome.

The study team also conducted an in vitro RT experiment, which showed that cell lysates from cells expressing RT of either type could cause reverse transcription of purified viral RNA from infected cells.

Viral N Sequences Discovered In The Human Cell Nucleus

Utilizing fluorescent in situ hybridization (FISH) technology, the study team pinned down the presence and ongoing transcription of the viral N sequences within the cell nucleus with the help of N-targeting fluorescent probes. The N sequences were found in the cytoplasm, as expected of cells infected by SARS-CoV-2.

But FISH also picked up N RNA signals from the nucleus of cells that overexpressed LINE-1, showing that integrated N sequences in the host genome were being transcribed there.

Importantly this occurred in about 35% of cells with overexpressed LINE-1, compared to 12% of non-LINE-1-overexpressing cells. Again, 30% of infected cells that were transfected by LINE-1 plasmids showed FISH nuclear N signals, but only 13% of non-transfected cells. About a tenth of infected non-transfected cells showed nuclear N signals, indicating endogenous RT activity.

Cytokines And LINE-1 Mediate Reverse Transcription

The study team found that published RNA sequencing data from SARS-CoV-2-infected cells showed a high number of LINE-1 elements, which was directly correlated with the abundance of chimeric reads. Within the Calu-3 cell line that allows efficient infection, a number of such elements were upregulated three- to four-fold following infection by the virus. PCR testing showed that these cells demonstrated RT-mediated integration of viral genomic material into the host DNA, perhaps by the activation of the LINE-1 RT.

Importantly, cytokines can also upregulate endogenous LINE-1 expression two- to three-fold.

Serious Implications

The study findings show induced LINE-1 expression in cells stressed by viral infection or exposed to cytokines, suggesting a molecular mechanism for SARS-CoV-2 retro-integration in human cells.

Importantly the integrated sequences are probably sub-genomic and cannot produce live infectious virions. (This needs to be verified by further studies).This explains the positivity of later PCR tests for viral RNA in clinically recovered patients.

However further detailed research will be needed to understand whether this will lead to the continuing expression of viral antigens capable of inducing an immune response. It is also possible that the presence of viral elements in the genome could exacerbate the deleterious aspects of the immune response, such as hyper-inflammation, mediated by excessive cytokine release, or autoimmunity.

The study team suggests that the site of insertion and regulation

by epigenetic factors, as well as the existing immune state of the patient, may affect the translation of these sequences and their possible clinical consequences. If retro-integration does affect the clinical severity and treatment of COVID-19, the same may possibly be true of other viruses, such as dengue or Zika virus as well, or even the influenza virus.

Also the study findings suggests that many PCR positive results could be due to viral transcripts from such chimeric sequences rather than reflecting the presence of replicating virus in the host. If validated, this will require better tests to be used when assessing the efficacy of COVID-19 therapies in clinical trials, for example, in the future.

This study findings only showed this integration in a lab dish, although it also cites published sequence data from humans infected with SARS-CoV-2 that suggest it has happened.

The study team emphasize that their results don't imply that SARS-CoV-2 establishes permanent genetic residence in human cells to keep pumping out new copies, as HIV does.

Dr Jaenisch told Thailand Medical News, "There are open questions that we'll have to address."

Medical And Research Community Abuzz With Study Findings And Reactions

Few veteran retrovirologists are fascinated while other scientists are divided about the importance of the new work and its relevance to human health, and some are harshly critical.

Professor Dr Robert Gallo, who heads the Institute of Human Virology said publicly, "This is a very interesting molecular analysis

and speculation with supportive data provided “I do not think it is a complete story to be certain but as is, I like it and my guess is it will be right.”

Professor Dr David Baltimore, a virologist at the California Institute of Technology who won the Nobel Prize for his role in discovering RT, describes the new work as “impressive” and the findings as “unexpected” but he notes that Dr Jaenisch and colleagues only show that fragments of SARS-CoV-2’s genome integrate.

Dr Baltimore told media, “Because it is all pieces of the coronaviral genome, it can’t lead to infectious RNA or DNA and therefore it is probably biologically a dead end. It is also not clear if, in people, the cells that harbor the reverse transcripts stay around for a long time or they die. The work raises a lot of interesting questions.”

Professor Dr Zandrea Ambrose, a retrovirologist at the University of Pittsburgh, adds that this kind of integration would be “extremely rare” if it does indeed happen. She notes that LINE-1 elements in the human genome rarely are active.

She said, “It is not clear what the activity would be in different primary cell types that are infected by SARS-CoV-2.”

Professor Dr Melanie Ott, a virologists who studies HIV at the Gladstone Institute of Virology and Immunology, says the findings are “pretty provocative” but need thorough follow-up and confirmation.

She said, “I have no doubt that reverse transcription can happen in vitro with optimized conditions.”

However she notes that SARS-CoV-2 RNA replication takes place in specialized compartments in the cytoplasm.

She added, “Whether it happens in infected cells and leads to significant integration in the cell nucleus is another question.”

Leading retrovirologist Professor Dr John Coffin of Tufts University calls the new work “believable,” noting that solid evidence shows that LINE-1 RT can allow viral material to integrate in individuals, but he’s not yet convinced. The evidence of SARS-CoV-2 sequences in individuals.

Dr Coffin says, “should be more solid,” and the in vitro experiments conducted by Dr Jaenisch’s team lack controls he would have liked to have seen. “All in all, I doubt that the phenomenon has much biological relevance, despite the authors’ speculation.”

Some critics on twitter said that the preprint’s conclusions are “a strong, dangerous, and largely unsupported claim.”

Dr Jaenisch however emphasizes that the paper clearly states the integration the study team think happens could not lead to the production of infectious SARS-CoV-2.

Dr Jaenisch says, “Let’s assume that we can really resolve these criticisms fully, which I’m trying to do. This might be something not to worry about.”

However other scientist are calling for more urgent studies as even integration of pats of the Virus RNA into the human genome can have varying implications and even change the course of medical sciences and human genetics and various cellular pathways.

Thailand Medical News will be providing updates on this new research and subsequent studies.

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