Current Human Embryonic Stem Cell Lines Contaminated With Potentially Dangerous Non-Human Molecule, UCSD/Salk Team Finds

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Currently available lines of human embryonic stem cells have been contaminated with a non-human molecule that compromises their potential therapeutic use in human subjects, according to research by investigators at the University of California, San Diego (UCSD) School of Medicine and the Salk Institute in La Jolla, California.

In a study published online January 23, 2005 in the journal *Nature Medicine*, the researchers found that human embryonic stem cells, including those currently approved for study under federal funding in the U.S., contain a non-human, cell-surface sialic acid called N-glycolylneuraminic acid (Neu5Gc), even though human cells are genetically unable to make it. In a related paper published November 29, 2004 by the *Journal of Biological Chemistry* (JBC), the Varki group has also discovered the exact cellular mechanism by which this occurs.

In studies with one of the federally approved human embryonic stem cell lines, the investigators determined that the Neu5Gc is incorporated by the stem cells when they are grown or derived from laboratory cultures that contain animal sources of the non-human Neu5Gc molecule. All traditional culture-dish methods used to grow all human embryonic stem cells include animal-derived materials, including connective tissue cells (so-called "feeder layers") from mice and fetal calf serum.

Ajit Varki, M.D. UCSD professor of Medicine, and cellular & molecular medicine

"The human embryonic stem cells remained contaminated by Neu5Gc even when grown in special culture conditions with commercially available serum replacements, apparently because these are also derived from animal products," said both papers' senior author Ajit Varki, M.D., UCSD professor of medicine and cellular & molecular medicine, and co-director of the UCSD Glycobiology Research and Training Center.

The research in *Nature Medicine* was done with human embryonic stem cells grown in the laboratory of Fred Gage, Ph.D., professor, Laboratory of Genetics, the Salk Institute, La Jolla, California, an adjunct professor of neurosciences at UCSD, and an author on the *Nature Medicine* paper.

Previously, the Varki lab found in 1998 that humans are uniquely different from other mammals studied in that people do not express Neu5Gc *****. In a 2003 study ******, the UCSD researchers found that humans have naturally occurring antibodies that are directed against Neu5Gc. In the current *Nature Medicine* paper, the scientists found that the human embryonic stem cells contaminated with Neu5Gc became, effectively, like animal cells, being attacked by human antibodies, and thus rendering them useless as a potential therapeutic tool in humans.

"It's an important safety issue because this opens up the idea that metabolic transfer of glycans is occurring between cells," said Gage. "Also, components of the growth medium have the capacity to change the immunological characteristics of the human ES cells. More research is needed to understand the optimal conditions for preparing human cells for therapeutic application."

"We considered that one partial solution to the problem was to use human serum in the growth medium," Varki said. When the team grew the cells in heat-inactivated human serum specially selected for low concentrations of anti-Neu5Gc antibodies, the immune response was significantly reduced, but not completely eliminated.

In their experiments, the researchers used recently developed probes to detect the presence of Neu5Gc on the cell surface of human embryonic stem cells that had been grown in traditional culture conditions. The scientists further confirmed the presence of Neu5Gc with a process called electrospray mass spectrometry. The percentage of total sialic acids present as Neu5Gc in the embryonic stem cells varied from 2.5 to 10.5 percent. In human embryonic stem cells that had been allowed to differentiate into embryoid bodies (EB), which is the first step in preparing them for potential use in humans, the percentage of total sialic acids present still ranged from 5 to 17 percent.

Varki and his team noted that many efforts have been made during the last few years to try to eliminate any animal-derived culture components in human stem cell culture. However, many of the specialized media used for growth and differentiation still contain materials from animal sources and are hence contaminated with Neu5Gc.

In addition to using human serum, the researchers suggested the possibility of using what are called "feeder cells" from mice with a human-like defect in Neu5Gc production. They noted that they have recently produced such a mouse. Another possibility being attempted by groups in other parts of the world is to use human embryo-derived connective tissue cells as the feeder layer in the culture.

A further solution might be a short-term culture in heat-inactivated serum from the actual patient who is going to receive the therapy, the scientists said. However, it may still prove difficult to completely eliminate the Neu5Gc, because is has become metabolically incorporated into the currently available, federally-funded human embryonic stem cell lines.

"With this discovery, the preexisting general concern about using animal products for deriving human embryonic stem cells has become more specific, being defined in molecular terms," Varki said.

"Such issues will, of course, become irrelevant if complete elimination of Neu5Gc can be achieved by deriving new human embryonic stem cells that have never been exposed to Neu5Gc-containing animal products of any kind," the researchers said in the *Nature Medicine* paper, noting that none of the suggested approaches guarantees the complete elimination of Neu5Gc from existing cultures. "Therefore, it would seem best to start over again with newly derived human embryonic stem cells that have never been exposed to any animal products, and ideally, only ever exposed to serum from the intended transplant recipient."

"However, such an approach could not be pursued under existing rules for the use of federal grant dollars," Varki said.

The first author of the *Nature Medicine* study is Maria J. Martin, Ph.D., a post doctoral researcher in Varki's lab at UCSD. An important additional author is Gage's post doctoral fellow Alysson Muotri, Ph.D. The study was funded by the National Institute of General Medical Sciences at the National Institutes of Health, the Lookout Fund, and by the G. Harold and Leila Y. Mathers Charitable Foundation of New York.

In addition to Varki, authors of the related study in *JBC* included Muriel Bardor, Ph.D. and Dzung Nguyen, Ph.D., post-doctoral fellows, and Sandra Diaz, a research associate. They determined that Neu5Gc gets into human cells by being engulfed in fluid droplets and then moved to the cytoplasm of the cell by a "pump" called the lysosomal sialic acid transporter.

Varki noted that this pathway is an unusual and previously unknown one that may also be relevant to the entry of other small molecules into cells. In addition, the *JBC* study showed how Neu5Gc attached to dietary proteins from animals could be incorporated into cells lining the stomach and colon, organs where consumption of red meat has been associated with risk of cancer.

"Knowing the mechanism that this molecule uses to get into human cells may give us clues to possible solutions to the problems that it may cause in various situations," Varki said.

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