Methods to multiply pluripotent cells for potential therapies raise worries about cancer

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The therapeutic promise of human stem cells is indisputably huge, but the process of translating their potential into effective, real-world treatments involves deciphering and resolving a host of daunting complexities.

Writing in the February 25 online issue of the journal *PLOS ONE*, researchers at University of California, San Diego School of Medicine, with collaborators from The Scripps Research Institute (TSRI), have definitively shown for the first time that the culture conditions in which stem cells are grown and mass-produced can affect their genetic stability.

"Since genetic and epigenetic instability are associated with cancers, we worry that similar alterations in stem cells may affect their safety in therapeutic transplants. Certain mutations might make transplanted stem cells more likely to form tumors, introducing the risk of cancer where it didn't exist before," said co-corresponding author Louise Laurent, MD, PhD, assistant professor and director of perinatal research in the Department of Reproductive Medicine at UC San Diego School of Medicine.

"This study shows the importance of quality control," added Jeanne F. Loring, PhD, professor and director of the Center for Regenerative Medicine at TSRI, and adjunct professor in the UC San Diego Department of Reproductive Medicine and the study's other co-corresponding author. "It's almost certain these cells are safe, but we

want to make sure they are free from any abnormalities."

To exploit the transformative powers of human pluripotent stem cells, which include embryonic stem cells and induced pluripotent stem cells, requires producing them in large numbers for transplantation into patients.

"During this culturing process, mutations can occur, and mutations that increase cell survival or proliferation may be favored, such that the cells carrying such mutations could take over the culture," said Laurent.

Human pluripotent stem cells are cultured in several different ways. Key variables are the surfaces upon which the cells are cultured, called the substrate, and the methods used to transfer cells from one culture dish into another as they grow, called the passage method.

Originally, scientists determined that stem cells grew best when cultured atop of a "feeder" layer that included other types of cells, such as irradiated mouse embryonic fibroblasts. For reasons not fully understood, these cells provide stem cells with factors that support their growth. However, concerns about the feeder cells also introducing undesirable materials into stem cells has prompted development of feeder-free cultures.

Moving cells from one culture dish to another has traditionally been done manually, with technicians physically separating the cultured cells into small clumps with an instrument. "It's very labor-intensive," said Laurent, "so new methods that use enzymes to separate individual cells were created."

In the *PLOS ONE* paper, Laurent and colleagues compared stem cells grown on two substrates (with and without feeder cells) and passaged using manual and enzymatic methods. They report that the use of

enzymes to passage the stem cells was strongly associated with increased genetic instability. Some of the mutations observed in the stem cells were previously known, but Laurent said others were seen for the first time, including deletion of a region of the genome that includes the gene P53, which is frequently deleted in cancer cells.

"I think these results call into question the use of enzymatic passaging, at least with enzymes that separate the cultures into single cells, for clinical use. However, we don't want to imply that any culture method is absolutely 'safe.' Any new culture method should be evaluated for its impact on genetic stability, and every batch of cells destined for the clinic should be tested using sensitive high-resolution methods for detecting genetic alterations.

"The processes used to maintain and expand stem cell cultures for cell replacement therapies need to be improved, and the resulting cells must be carefully tested before use."

Provided by University of California - San Diego