Study of stem cell diseases advanced by new technique

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A rare genetic disease called dyskeratosis congenita, caused by the rapid shortening of telomeres (protective caps on the ends of chromosomes), can be mimicked through the study of undifferentiated induced pluripotent stem cells, according to new findings from the Stanford University School of Medicine. Although dyskeratosis affects only about one in a million people, the scientists' findings could greatly facilitate research into this and other diseases caused by stem cell malfunctions, including some bone marrow failure syndromes and, perhaps, pulmonary fibrosis.

The study, which used iPS cells created from the cells of patients with dyskeratosis, explains why sufferers experience a wide variety in the types and severity of symptoms, ranging from abnormal skin pigmentation and nail growth to lung scarring, bone marrow failure and cancer. The key lies in the activity of telomerase, an enzyme critical to aging and cell renewal.

"We were very surprised to find such a clear correlation between the quantity of functional telomerase, the severity of the cellular defect and the severity of the patient's clinical symptoms," said associate professor of medicine Steven Artandi, MD, PhD. "Our work suggests that, in patients with dyskeratosis congenita, tissue stem cells are losing their ability to self-renew throughout the body. This is a new, unifying way to think about this disease, and it has important implications for many other conditions."

Unlike embryonic stem cells, which they closely resemble, iPS cells are created by tinkering with the genetic program of cells from adult tissue, such as skin. In most disease models, the cells are then coaxed to differentiate, or specialize, into a specific type of tissue for further study. However, in this study the cells remained undifferentiated to allow scientists to assess the activity of telomerase, which is prevalent in stem cells, but expressed at very low levels in most non-stem cells. The findings in this study are the first to illustrate the usefulness of non-specialized iPS cells in the study of a human stem cell disease.

Artandi, who is also a member of the Stanford Cancer Institute, is the senior author of the study, which will be published online May 22 in *Nature*. Postdoctoral scholar Luis Batista, PhD, is the first author. Artandi and Batista collaborated with professor of obstetrics and gynecology Renee Reijo Pera, PhD, a member of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, on the work.

Telomerase is a large complex of proteins that is active in cells that divide frequently, like tissue-specific stem cells and those of the immune system. It adds short DNA caps called telomeres to the ends of chromosomes. In most adult cells, the telomeres become shorter with each round of cell division; when the telomeres reach a certain minimum length, the cell can no longer divide. Stem cells and others that must divide often circumvent this "molecular clock" by keeping telomerase on hand.

Researchers have known for some time that patients with dyskeratosis congenita have shorter-than-normal telomeres. They also often have one or more mutations in the genes that encode the proteins that make up the telomerase complex. But it's not been clear why some people are affected much more severely than others — some have only low blood counts, while others progress to severe bone marrow failure, develop pulmonary fibrosis, show defects in skin and nails and have drastically

shortened life spans. Because so few cells in the adult body express telomerase, it's been extremely difficult to get enough of the complex to study — until now.

In the current study, Artandi and Batista created iPS cells from the skin cells of five patients with varying severities of dyskeratosis congenita. They then grew a sufficient amount of the undifferentiated cells in the laboratory to be able to directly compare their levels of telomerase activity and telomere lengths. (Although mature skin cells don't express telomerase, the protein complex is activated during the reprogramming that transforms skin cells to iPS cells.)

They found that the cells from patients with a mutation in one of the two copies of the gene for the telomerase workhorse protein — who typically had the less-severe clinical symptoms — had levels of telomerase activity that were about 50 percent of normal. In contrast, iPS cells from male patients with a mutation in a related gene on the X chromosome (of which they have only a single copy) displayed telomerase activity levels that were about 5 to 15 percent of normal. The two patients with mutations in this gene experienced the full panel of symptoms, from epidermal involvement to bone marrow failure, and had very short telomeres.

"We found that if we continued to grow those iPS cells in culture for many weeks, they suddenly lost their ability to self-renew," said Artandi. "Their short telomeres induced differentiation and caused a loss of stem cell characteristics."

The researchers concluded that the symptoms seen in patients with dyskeratosis congenita are likely to be caused by the gradual loss of tissue-specific stem cells in the skin, bone marrow and other organs. Without these stem cells, the body can't replenish damaged or developing tissues.

"This is very similar to what happens as we age," said Artandi. "Our telomeres grow shorter and this may cause the same loss of self-renewal in our tissue stem cells. Telomere shortening is also seen in most human cancers, so studying iPS cells may provide new insights into cancer and aging, as well as provide a system to develop novel therapeutics."

Learning more about how telomerase affects self-renewal may lead not only to new therapies for dyskeratosis congenita, but also to new insights about aging and disease, the researchers believe. They are continuing to make new iPS cell lines from patients with other forms of dyskeratosis congenita to further advance their work into understanding cellular self-renewal.

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