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Team identifies strategy to reverse the disease dyskeratosis congenita

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Dyskeratosis congenita, or DC, is a rare, inherited disease for which there are limited treatment options and no cure. Typically diagnosed in childhood, the disorder causes stem cells to fail, leading to significant problems including bone marrow failure, lung fibrosis, dyskeratosis of the skin and intestinal atrophy and inflammation. Patients are also at heightened risk of several types of cancer.

A common underlying feature of the disease is the presence of shortened telomeres. Telomeres are the structures that protect, or "cap," the ends of chromosomes, but they tend to shorten with cell division and age, and can thus lose their protective functions. Many DC patients have a mutation in the DKC1 gene, which codes for a component of the enzyme called telomerase that helps maintain telomere length. Because telomerase is most essential in tissues that divide frequently, notably, epithelial tissues such as the skin, gut and lungs, this is where defects crop up in these individuals.

In a new study published in *Cell Stem Cell*, researchers from the University of Pennsylvania led by Christopher J. Lengner of the School of Veterinary Medicine and Brad Johnson of the Perelman School of Medicine have found a link between telomeres and a molecular signaling cascade called the Wnt pathway that may point to a treatment option for DC patients. The link was identified initially using mice in Johnson's lab, and together the Lengner and Johnson groups extended these findings to human tissues in culture to demonstrate that stimulating the Wnt pathway reversed signs of DC-related dysfunction in the intestines.

Their study underscores the presence of a positive feedback between Wnt signaling and telomere function, whereby stimulation of Wnt signaling activates expression of proteins that enhance the "cap" on telomeres, leading to more functional stem cells, which in turn stimulates the Wnt pathway further.

The research also provides insights into strategies to combat telomere dysfunction, a process that has also been implicated in some cancers and natural aging.

"What you see in the mutant mice and the dyskeratosis congenita patients is the consequence of having less telomerase activity in rapidly dividing tissues," said Johnson, co-senior author on the study and an associate professor of pathology and laboratory medicine in Penn Medicine. "It's not the same thing as natural aging where you might have telomere defects in cells that don't have a lot of telomerase activity to begin with. But I think it's fair to say our findings inform an understanding of some of what might be happening as telomeres shorten in aging."

Lengner, an assistant professor in the Department of Biomedical Sciences in Penn Vet, was co-senior author on the study.

"Right now the main therapy for these patients is a bone marrow transplant," Lengner said. "That can address the bone marrow failure but doesn't fix other problems associated with the disease, and especially not the risk of cancer. This work suggests a way to address the underlying cause of the disease."

Earlier research with mouse models of DC had suggested that there might be a connection between the Wnt pathway and telomerase. And a recent study in DC patients' cells found a decrease in activity in the Wnt pathway. So the Penn researchers wanted to explore whether activating

Wnt could reverse the effects of the disease.

To do so, the team used three nascent techniques. First, they employed induced pluripotent stem cells, or iPSCs, which are adult cells that are "reprogrammed" to closely resemble embryonic stem cells. This allowed the researchers to take cells from DC patients, as well as from healthy individuals, and transform them so the cells, like stem cells, could give rise to many different cell types.

Second, they used the CRISPR/Cas9 system, a straightforward method with dramatic effects. Like a cut-and-paste for the genome, it creates double-strand breaks at a particular site in the DNA and then repairs it, incorporating a new, desired sequence, using homologous recombination. The researchers used this gene-editing system to introduce a DKC1 mutation into the healthy human iPSCs, and also to correct the disease-causing mutation in the iPSCs from DC patient samples. This way, they had matched sets of cell lines to test, which could rule out the effects of any background genetic variation.

Finally, the researchers grew what are known as "organoids," a growth in vitro that resembles an organ, through a process called directed differentiation, in which the iPSCs were fed certain molecules that mimic the signals that stem cells receive in normal development. In this case, the cells were coaxed to form a human intestinal organoid, which naturally forms a tube-like structure, recapitulating the tubes of the human gastrointestinal system.

When the Penn team observed the development of the intestinal organoid, they found that initially, the DC patients' cells seemed to form normally.

"But at the time point when they're supposed to make intestines and form a gut tube, things fell apart," Lengner said.

While the healthy patients' samples and the samples from DC patients that had been corrected by CRISPR formed a tube, the original patients' cells and the cells that had had the disease mutation introduced by CRISPR appeared to follow a normal course of development for several days, but by two weeks lacked the tube-like structure seen in the healthy samples and the disease-corrected samples. These cells also had shorter telomeres, with the intestinal organoids from DC patients' having the shortest of any cell type.

"We could see at the molecular level that this is accompanied by a failure to activate specific intestinal stem cell gene programs," Lengner said, "specifically genes in the Wnt pathway."

The next logical step for the researchers was to activate Wnt to see if these defects could be reversed. They treated organoids derived from DC patient iPSCs with a compound called CHIR that stimulates the Wnt pathway, and found that it restored the formation of the tube-like structure as well as intestinal stem cell gene expression. The treatment also increased telomerase activity and telomere length in the cells with a mutant DKC1.

To assess this treatment approach in a more clinically relevant model, they transplanted the human intestinal organoids into mice. Mice that received a transplant containing the DKC1 mutation and were also treated with an FDA-approved stimulator of the Wnt pathway, lithium, maintained their intestinal tissue structure and had high expression of Wnt target genes. In effect, they resembled the mice that received a transplant of an organoid derived from a healthy patient.

The study, the researchers said, offers proof of principle that activating the Wnt pathway can reverse at least the gastrointestinal phenotypes associated with dyskeratosis congenita. Looking ahead, they would like to try accomplishing the same feat in other tissue types affected by the

disease, such as the lungs and even the blood.

And while lithium is an approved drug, it is not selective for the Wnt pathway and has side effects, and thus "screening for additional therapeutic agents might be beneficial," Johnson said.

Provided by University of Pennsylvania