Study shows immune system protein involved in reprogramming adult cells to express stem cell genes

December 22 2009, BY KRISTA CONGER

(PhysOrg.com) -- Scientists have discovered a protein required to quickly and efficiently reprogram human skin cells to express embryonic stem cell genes.

Scientists believe there is much promise for induced pluripotent stem cells: normal adult cells that have been manipulated to develop the stem-cell-like ability to differentiate into other types of cells, potentially to be used to repair damaged tissue and treat the ravages of disease.

But making these so-called iPS cells is both time-consuming and inefficient.

Now researchers at Stanford's School of Medicine have discovered a protein required to quickly and efficiently reprogram human skin cells to express embryonic stem cell genes. The finding could eliminate a major bottleneck in the generation of iPS and embryonic stem cells — that of removing molecular tags called methyl groups from specific regions of cellular DNA. Without this process of demethylation, the stem cell genes are silent in adult, or differentiated, cells.

"The mechanism of DNA demethylation in mammals has eluded us for decades," said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor and member of Stanford's Institute for Stem Cell Biology and Regenerative Medicine. "Now we've identified a protein involved in

targeted DNA demethylation, and we've also shown that it's critically important in reprogramming adult cells to function more like their stem cell predecessors." Blau is the senior author of the research, published online Dec. 21 in Nature.

The researchers used a high-efficiency cell fusion strategy to identify the protein, called activation-induced cytidine deaminase, or AID. This protein is most commonly known for its involvement in the immune system in generating germ-fighting antibodies. But Blau and her colleagues uncovered an unexpected presence — and an unanticipated role — of the protein in embryonic stem cells.

The researchers anticipate that harnessing the activity of AID and other proteins with which it may work could revolutionize the generation of iPS cells. It may also help in creating embryonic stem cells through a process called somatic cell nuclear transfer. Both methods rely on the removal of the molecular tags from specific regions of DNA.

While iPS cells share some similarity with embryonic stem cells, they differ in important respects. They are usually generated by taking a specialized adult cell and inducing the expression of embryonic-stem-cell-specific genes. After two to three weeks, a small proportion (fewer than one in 1,000) of the cells begin to look and act like embryonic stem cells. The process is slow and inefficient in part because the DNA in the adult cells must be reprogrammed, by removing and adding methyl groups across the genome, in order to switch off any genes that maintained the cells' previously specialized state.

However, iPS cells are useful because they allow the study of cells that can become a variety of tissues (meaning that they are pluripotent) while bypassing many of the ethical hurdles associated with true embryonic stem cells. They also offer a way to make patient-specific stem cells that could potentially be used for therapy many types of disorders and for

modeling human diseases in the laboratory.

Blau, research scientist Nidhi Bhutani, PhD, and graduate student Jennifer Brady developed a way to analyze how directed nuclear reprogramming occurs. They fused mouse embryonic stem cells with human adult skin cells to create cells that contain DNA from both species. Although the cellular factors mingle freely, differences in the gene sequences between the two species allowed the researchers to identify which gene products originated from the human nucleus. Within days of fusion, they found that about 70 percent of the cells began to turn on human pluripotency genes.

Something in the mouse embryonic stem cells was inducing a rapid change in the DNA methylation status of these human skin cells. Blau wondered if AID from the mouse embryonic stem cells could be the cause. Although AID is primarily known as an immune system molecule, it had recently been shown to be present at low levels in mammalian germ cells. Germ cells generate the egg and sperm that, when combined, will become the ultimate in pluripotent stem cells: a fertilized egg. A recent study in zebrafish also suggested it might be involved in non-specific DNA demethylation after fertilization.

Sure enough, they found that when they blocked AID expression in the fused cells, the expression of the human pluripotent genes dropped to nearly nothing. Adding it back restored the genes' expression. The methylation status of the DNA around the genes mirrored the expression patterns: Silent genes were methylated and active genes were unmethylated. Finally they showed that AID bound to methylated but not unmethylated regions of the genes.

"It's clear that AID plays a crucial role in directed reprogramming of cells to pluripotency," said Blau. "Most of our previous efforts to study DNA demethylation in mammals have resulted in very subtle changes in

methylation status. This isn't subtle." Unlike the current methods for generating iPS cells, it also doesn't require DNA replication or cell division and so may more closely mirror what happens in vivo when germ cells generate pluripotent gametes.

The researchers are currently testing to see if adding AID to adult mouse cells can in fact convert them to iPS cells more efficiently, or without requiring the addition of other pluripotency genes. They are also working to identify other AID-associated factors that might be involved in the demethylation process.

Provided by Stanford University Medical Center