# Induced pluripotent stem cell retain an inactivated X chromosome

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Female induced pluripotent stem (iPS) cells, reprogrammed from human skin cells into cells that have the embryonic-like potential to become any cell in the body, retain an inactive X chromosome, stem cell researchers at UCLA have found.

The finding could have implications for studying X chromosome-linked diseases such as Rett syndrome, caused by mutations in a gene located on the X chromosome.

The findings differ from those seen in mouse skin cells that are reprogrammed into iPS cells, in which the inactive X chromosome reactivates, said Kathrin Plath, senior author of the study and a scientist with the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA.

"We knew from our studies that in reprogrammed mouse cells, the X chromosome becomes active again," said Plath, an assistant professor of biological chemistry and one of the first scientists in the world to reprogram mouse and human adult cells into iPS cells. "The question we wanted to ask is what happens in female human iPS cells."

All female cells have two X chromosomes - one from each parent - and in early development, one X chromosome is permanently inactivated. The inactivation of the X chromosome ensures that females, like males, have one functional copy of the X chromosome in each cell of the body and that the cells develop normally.

Plath and her team took human skin cells from females of varying ages. The cells have one active and one inactive X chromosome. The research team added four transcription factors to reprogram the cells into iPS cells and examined the resulting iPS cells to uncover the status of the X chromosome. They found that one X chromosome remained inactive, making the reprogrammed cells similar to most female human embryonic stem cells, which have one active and one inactive X chromosome. More than 30 different iPS cells lines were analyzed in the study, with the same result, Plath said.

'The presence of the inactive X chromosome in the iPS cells raised the question of which of the two X chromosomes is inactive in the iPS cell lines," Plath said.

During mouse and human embryonic development, one X chromosome is silenced in every female somatic cell, but the selection of either the paternal or maternal chromosome for silencing is random in each cell. A typical population of skin cells is mosaic for which X chromosome is silenced, containing about 50% of cells that inactivated the paternally inherited X, while the other 50% of cells inactivated the maternal X. Plath and her team sought to determine whether X chromosome silencing in iPS cell populations was random as well.

Plath found that all cells in the same reprogrammed iPS cell line exclusively expressed the same X chromosome and had the other X inactive. Also, Plath found that different iPS cells lines that came from the same adult skin cell population can differ in which X chromosome is inactivated.

Because the inactivated X is retained during human cell reprogramming and differentiation of iPS cells, these cells are well positioned for the study of X-linked diseases because it would be possible to get lines either expressing the normal or mutant allele from the same female patient.

"This non-random pattern of X chromosome inactivation found in iPS cell lines has critical implications for clinical applications and disease modeling and could be exploited for a unique form of gene therapy for X-linked diseases," Plath said.

In collaboration with other stem cell researchers at

UCLA, including Bill Lowry and April Pyle, they generated isogenic, or identical genetically, female iPS cell lines from females who are carriers of the mutation in the dystrophin gene on the X chromosome, making themcarriers for muscular dystrophy (DMD). They isolated iPS cell lines that either exclusively expressed the normal or the mutant version of the affected dystrophin gene responsible for DMD. These cells are isogenic and represent the perfect pair of control and mutant cell types for investigation of the disease phenotype. They currently are studying the effect of the mutation in muscle differentiation.

This observation could also be useful in potential cell therapy for X-linked disorders such as Rett Syndrome as the differentiated cells derived from iPS cells expressing the normal protein could possibly be transplanted back in place of those expressing the mutant protein.

"For studies of X-linked diseases with female iPS cells, one needs to be careful about which X chromosome is expressed," Plath said.

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