NCCS finds dual mechanism to help embryonic stem cells maintain pluripotency

Our work will be helpful in regenerative medicine, says Deepa Subramanyam (right). | Photo Credit: Special Arrangement

Embryonic stem cells are capable of generating all the cell types that compose the organs and systems of the human body. Now, researchers at Pune's National Centre for Cell Science (NCCS) have found a dual mechanism that keeps specific genes off, which helps the embryonic stem cells maintain pluripotency — their ability to give rise to all the cell types. The dual mechanism functions in such a way that even if one mechanism fails, the other can function as a back-up and help the embryonic stem cells maintain pluripotency.

Embryonic stem cells contain multiple endocytosis-associated genes whose expression is suppressed unlike in the case of the specialised or differentiated cells. Some of the genes are directly responsible or regulate the transport of molecules which are present on the cell surface membrane to the interior of the cell. The precise mechanism by which the expression of endocytosis-associated genes are turned off in embryonic stem cells and the role of endocytosis (transport of molecules from the membrane surface to inside the cell) in maintaining pluripotency was not known. A team led by Dr. Deepa Subramanyam from NCCS has deciphered them. The results were published in the journal *Scientific Reports*.

"We attempted to identify and ascertain if certain genes that are associated with endocytosis have their expression kept under check or not in stem cells, and if these genes had any role in maintaining the stemness of embryonic stem cells," says Dr. Subramanyam.

The team identified two pathways — polycomb repressive complex (PRC2) and embryonic stem cell-specific cell cycle (ESCC) regulating microRNAs — that suppressed the expression of the endocytosis-associated genes in embryonic stem cells but not in cells that have already differentiated. While the expression of 50 endocytosis-associated genes is kept under check by one pathway (PRC2), the expression of a smaller subset of 12 genes is also reduced by the action of the second pathway (ESCC).

The PRC2 complex has four subunits, and when one particular subunit (Ezh2) was knocked down it led to significant increase in the expression of endocytosis-associated genes. Similar results were obtained when another subunit (Suz12) was knocked down.

Stem cells have a class of small non-coding microRNAs called the ESCC-family of microRNAs. The microRNAs work by binding to the complementary sites seen on messenger RNA (mRNA). "Of the 50 endocytosis-associated genes, 21 genes had complementary sites for the microRNA, indicating that these 21 could potentially be controlled by microRNAs," she says.

"The function of the PRC2 complex is to suppress the expression of the 50 endocytosis-associated genes. And the microRNAs function as a back-up, in case the expression of some of the genes is not completely shut down by the action of the PRC2 complex," says Dr. Subramanyam. The stem cells will continue to exhibit pluripotency as a long as the expression of the endocytosis-associated genes is turned off.

To confirm whether the genes have to necessarily be turned off for pluripotency to be maintained in stem cells, the researchers introduced the genes into stem cells in such a manner that the expression of these genes was not turned in the stem cells. "We introduced only one gene at a time and we tested a total of two genes. In both cases, the embryonic stem cells began losing their pluripotency and there was an upregulation of differentiation markers," says Ridim Dadasaheb

Mote from NCCS and first author of the paper.

“Our work will be helpful in regenerative medicine. Understanding the pathways and mechanism of endocytosis can now give us a handle to try and convert induced pluripotent stem (iPS) cells, which are pluripotent, into specialised cells such as neurons by altering the expression of the endocytosis-associated genes,” says Dr. Subramanyam.

Fossilised bird dung from almost 1500 years ago was used for the study.

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