Researchers improve method to create induced pluripotent stem cells

July 20 2011

University of Minnesota Medical School researchers have developed a new strategy to improve the development of induced pluripotent stem cells (iPS).

Currently, iPS cells are created by introducing four defined genes to an adult cell. The genes reprogram the adult cell into a stem cell, which can differentiate into many different types of the cells in the body. Typically, the four genes introduced are Oct4, Sox2, Klf4 and c-Myc, a combination known as OSKM.

The U of M researchers found that by fusing two proteins – a master stem cell regulator (Oct4) and a fragment of a muscle cell inducer (MyoD) – they succeeded in "powering up" the stem cell regulator, which can dramatically improve the efficiency and purity of reprogrammed iPS cells.

"Our team discovered that by fusing a fragment of the powerful protein MyoD to Oct4 we could create a 'super gene' which would improve the iPS reprogramming process," said senior author Dr. Nobuaki Kikyo, Stem Cell Institute researcher and University of Minnesota Medical School associate professor. "The result is what we termed M3O, or 'super Oct4' – a gene that improves the creation of iPS cells in a number of ways. In the process we shed new light on the mechanism of making iPS cells."

The challenge with the previous method – OSKM – has been that very

few cells actually become iPS cells during reprogramming. In fact, the rates currently stand at about 0.1 percent. Another issue has been tumor development. Because some of the reprogramming genes introduced are oncogenes, the risk of developing tumors grows.

The research, led by Kikyo and Dr. Hiroyuki Hirai, both from University of Minnesota Medical School and Stem Cell Institute, led to a new gene model that minimizes such complications while amplifying the benefits of the process.

According to Kikyo, the new gene model – called M3O-SKM – improves iPS development by:

• Increasing efficiency. The efficiency of making mouse and human iPS cells was increased over 50-fold compared with the standard OSKM combination.

• Increasing purity. The purity of the iPS cells was much higher with the M3O-SKM gene introduction (98% of the colonies) compared with OSKM (5%).

• Facilitating the reprogramming. iPS cell colonies appeared in around five days with M3O-SKM, in contrast to around two weeks with OSKM.

• Decreasing the potential for tumor formation. M3O achieved high efficiency of making iPS cells without c-Myc, an oncogene that can potentially lead to tumor formation.

In addition, human iPS cells usually require co-culture with feeder cells typically prepared from mouse cells, obviously creating a problem when the cells are destined for human transplantation. The M3O model did not require such feeder cells, greatly simplifying the process.

The new process is outlined in the latest issue of the journal *Stem Cells*.

Future Impact

According to senior author Kikyo, this new strategy will dramatically speed up the process of making patient-specific iPS cells, which makes clinical applications via transplantation of the cells more feasible to treat many diseases incurable otherwise.

Many researchers are also examining how to reprogram one cell type into another without going through iPS cells; for instance, coaxing skin cells into becoming neurons or pancreas cells by introducing several genes.

The approach, called direct reprogramming, is thought to be the next generation approach beyond iPS cell technology.

The U of M approach – fusing a powerful protein fragment to other host proteins – can be widely applied to the direct reprogramming approach as well.

Provided by University of Minnesota