Novel reprogramming factor yields more efficient induction of human pluripotent stem cells

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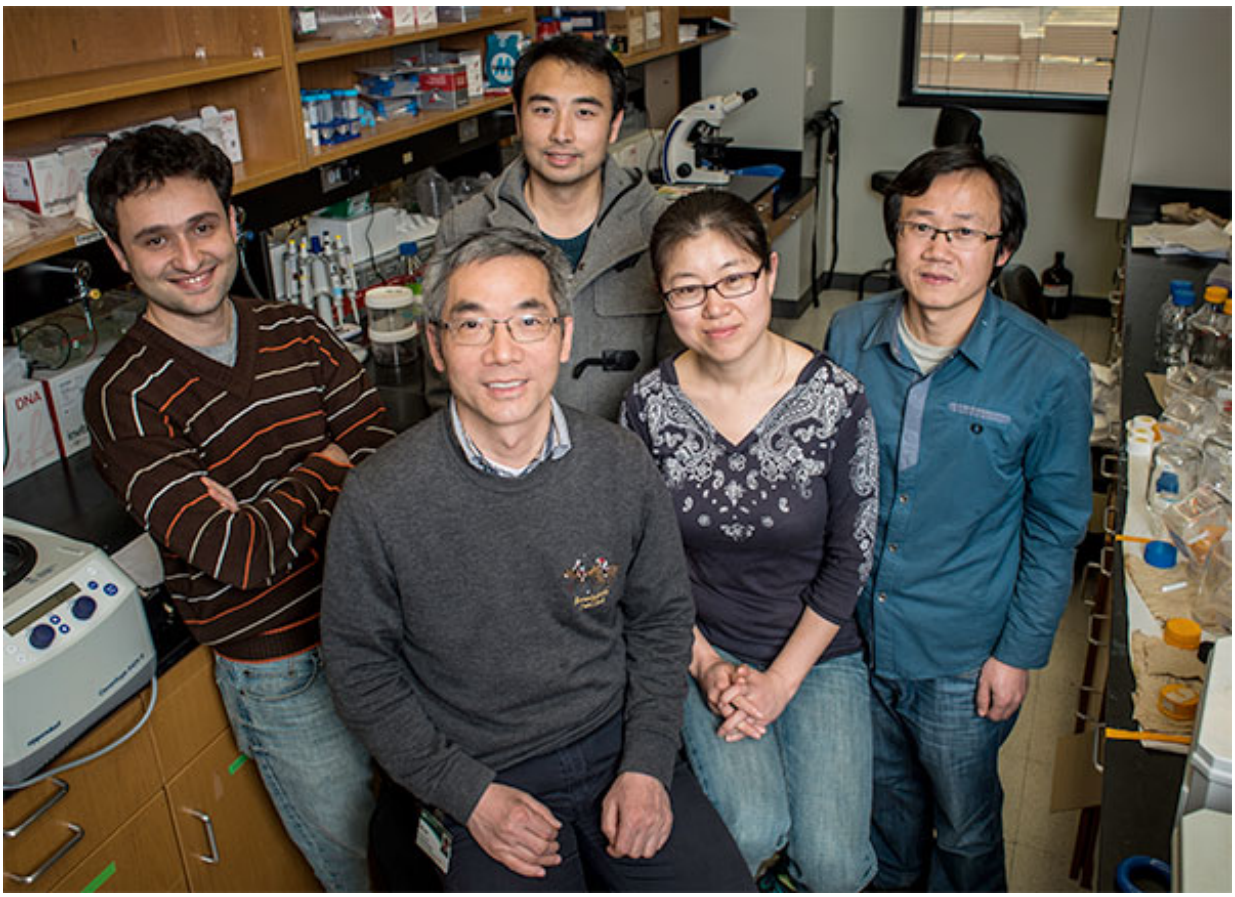

Front row from left: Kejin Hu, Ph.D., Chunping Yao, M.D., and Weihua Xu, Ph.D.; back row, Alireza Khodadadi-Jamayran and Ruowen Zhang, Ph.D.

Kejin Hu, Ph.D., of the University of Alabama at Birmingham, has found a robust reprogramming factor that increases the efficiency of creating human induced pluripotent stem cells (HiPSCs) from skin fibroblasts more than 20-fold, speeds the reprogramming time by several days and enhances the quality of reprogramming.

HiPSCs are believed to hold great promise for medical research and disease treatments. They are man-made versions of human embryonic stem cells (hESCs). Biomedical researchers can generate HiPSCs from many somatic cells such as fibroblasts from a skin biopsy, without destroying any human embryo. Like hESCs, HiPSCs have the ability to differentiate into any type of specialized cell. Thus, they can differentiate into any of the 200-plus different types of human cells. These cells are potent tools for drug development and modeling of disease, and they also hold potential to transform transplantation medicine by creating patient-specific pluripotent stem cells for cell-replacement therapies. Targets may include neurological diseases, heart ailments, blood diseases and diabetes. But this promise of iPSCs is hobbled by a low efficiency, less than 0.1 to 1 percent, in producing these cells, along with many other significant hurdles.

In a paper published March 7 in *Nature Communications*, Hu and fellow UAB researchers describe their successful hunt for a reprogramming factor that boosts the efficiency and shortens the time the cell takes to reprogram. The reprogramming factor is a kinase-family protein called BRD3R that reads acetylated histone codes in the chromosome.

When the Hu lab looked at the gene expression induced by overexpression of BRD3R during the reprogramming of human fibroblasts into iPSCs, they also solved a mystery that had endured half a century since 1962, when the Nobel Laureate John Gurdon first cloned a frog by placing the nucleus of an intestinal cell into an enucleated frog egg cell—namely, why does animal cloning succeed only with oocytes

that are in the metaphase II stage of mitosis? Hu found that the BRD3R gene, when introduced into fibroblasts, upregulated 128 mitotic genes, which yields a molecular insight into the mitotic advantage of reprogramming.

"When we saw the mRNA sequencing data, they explained why mitosis is so important in reprogramming," Hu said. "I was excited when we found a new reprogramming factor and also by this second discovery."

To reprogram the fibroblasts, the Hu lab used BRD3R delivered by a lentiviral vector, along with three of the genes that the Japanese Nobel Laureate Shinya Yamanaka used in his first successful reprogramming of mature mouse cells into iPSCs in 2006—OCT4, SOX2 and KLF4. BRD3R was unable to replace any of the three essential Yamanaka reprogramming factors, indicating that BRD3R has a distinct role from them in reprogramming. Hu and his colleagues did their reprogramming of the human somatic cells in xeno-free media, without using mouse feeder cells or serum from any sources. Xeno-free production will be an FDA good manufacturing practice requirement for future clinical use of human iPSCs.

Hu started his search for a new reprogramming factor after arriving at the Stem Cell Institute, UAB Department of Biochemistry and Genetics, in 2011. "I believed that new reprogramming factors would produce more authentic iPSCs in a more efficient, faster way."

He acquired a library of 558 human kinase genes and began to screen a batch of the first 89. After introducing the three Yamanaka genes and his test kinase genes into fibroblast cells, he looked for an increase in efficiency in iPSC generation, as judged by increased colony numbers of the successfully reprogrammed pluripotent cells that express pluripotent markers alkaline phosphatase and TRA-1-60. Hu's primary screen revealed 11 candidates, but only BRD3R acted as a new reprogramming

factor in a more rigorous secondary screen.

None of the BET subfamily genes similar to BRD3R—BRD2, BRD3 and BRD4—showed reprogramming activity. The iPSCs generated by BRD3R were shown to be pluripotent by the standard criteria. Although a member of the kinase library, BRD3R has not yet been demonstrated to have a kinase activity. But among the genes most consistently upregulated by BRD3R in the early stages of reprogramming were those encoding four kinases, including a master mitotic kinase and a critical mitotic kinase, five genes that regulate kinase activities, and one phosphatase gene. Therefore, Hu and colleagues write, "Even though BRD3R may not have a kinase activity, it appears to regulate an important mitotic kinase network to promote reprogramming."

In another paper published at the same time in *Stem Cells and Development*, Hu's lab reports a promising solution to an issue associated with the clinical application of human iPSCs. HiPSCs possess rampant growth like cancer cells, and the contamination of a single HiPSC cell in HiPSC-based transplants will pose a risk of tumor development in recipient patients. In addition, incompletely differentiated HiPSCs are believe to be tumorigenic as well. With HiPSC-based cell therapy entering into clinical trials already, there is no safety protocol to eliminate the risk of tumor development in patients receiving HiPSC-based transplants. One goal of Hu's research is to establish protocols to eliminate the tumorigenic cells in HiPSC-based transplants. His laboratory discovered that targeting an HiPSC marker surface protein PODXL using a well-established antibody kills pluripotent cells.

"We suggest," the authors write, "that the antibody could be employed to eliminate the tumorigenic pluripotent cells in human PSC-derived cells for cell transplantation."

PODXL is known to be involved in more than 10 different human

malignancies and has a reported role in metastasis and tumor invasion. Hu's laboratory is interested in finding whether the same antibody can kill other malignant cells in cancer patients.

Hu and colleagues found that PODXL has high gene expression in HiPSCs, and it is downregulated when the cells differentiate. They recently found that a residual PODXL-positive population persists, even after extended differentiation. Hu said it is urgent to examine the tumorigenicity of this residual PODXL-positive cell population in HiPSC-based transplants.

More information: Zhicheng Shao et al. The acetyllysine reader BRD3R promotes human nuclear reprogramming and regulates mitosis, *Nature Communications* (2016). DOI: 10.1038/ncomms10869

Provided by University of Alabama at Birmingham