Proteins that control energy use necessary to form stem cells

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Julie Mathieu (left), Hannele Ruohola-Baker, and Zsuzsa Agoston go over laboratory research results.

Proteins that regulate energy metabolism are essential for stem cell formation, University of Washington researchers find.

Two proteins that control how cells metabolize glucose play a key role in

the formation of human stem cells, UW researchers report.

The findings advance scientists' understanding of stem cell development but also suggest that the proteins, which also play a role in the process that transforms normal cells into cancer stem cells, might also be targets for new cancer therapies, the researchers write.

The findings appear online in the journal *Cell Stem Cell*. The paper's lead authors are Julie Mathieu, a post-doctoral fellow at the UW and Wenyu Zhou who was a graduate student at UW and is now a postdoctoral scholar at Stanford University, Department of Genetics. Dr. Hannele Ruohola-Baker, UW professor of biochemistry, is the paper's senior author.

In the study, the researchers induced mature human tissue fibroblasts to revert to an earlier stem cell-like state by inserting genes for four proteins, a process called reprogramming.

These reprogrammed cells have the extraordinary ability to develop into any type of cell in the human body, a capacity called pluripotency, and it is hoped that induced-pluripotent stem cells will one day be able to be used to create new tissues and organs to repair and replace those damaged by injury and disease.

Researches have known for some time that during reprogramming, cells must go through a stage in which they shut down metabolic pathway that they use to generate energy from glucose that requires the presence of oxygen in mitochondria, the cell's powerhouse and shift over to another pathway, called the glycolytic pathway, that generates less energy but does not require the presence of oxygen.

This shift may take place because in nature, embryonic and tissue stem cells often must survive in low-oxygen, or hypoxic, conditions.

This transition to a glycolytic state is of particular interest to cancer researchers as well, since as normal cells are transformed into cancer cells, which in many ways resemble stem cells, they, too, go through a glycolytic phase.

In their study, the UW researchers focused on the function of two proteins: hypoxia-induced factor 1α and 2α, or HIF1α and HIF2α. These proteins are transcription factors which mean they affect the regulation of a number of genes, allowing them to dramatically alter a cell's behavior. The researchers showed through loss-of-function analysis that each protein, HIF1α as well as HIF2α is required for generation of stem cells through reprogramming.

To tease out the impact of HIF1α and 2α on cellular processes in more detail, they stabilized the proteins in an active form and tested what each protein could do alone. They found that when HIF1α was stabilized, the cells went into the glycolytic state and produced more induced pluripotent stem cells than normal.

However, when they just activated HIF2α, they found the cells failed to develop into stem cells. "This was a big surprise," said Mathieu. "These proteins are very similar but HIF1α gives you lots of stem cells; HIF2α, none."

If stabilized together, HIF2α won the battle, repressing all stem cell formation.

Further investigation found that HIF2α does indeed promote the shift to glycolysis in an early stage of the cells' reprogramming but if it persists too long has the opposite effect, blocking the progression to the stem cell state.

"HIF2α is like Darth Vader, originally a Jedi who falls to the dark side,"

said Ruohola-Baker. "While HIF1α, the good guy is beneficial for reprogramming throughout the process, HIF2α, if not eliminated, turns bad in the middle and represses pluripotency."

HIF2α does this in part by upregulating the production of another protein, called TRAIL, for TNF-related apoptosis-inducing ligand, that is known to, among other things, cause tumor cells to self-destruct through a process called apoptosis.

Zhou said the findings suggest that there may be other proteins in this protein family that are playing alternating "good guy/bad guy" roles during stem cell development. "It is very intriguing that HIF2α has the capacity to both promote and repress pluripotency, doing so at different stages in a cellular reprogramming process," she said.

The findings have several implications for stem cell research, says Mathieu: first, they indicate that it may be possible to use HIF1α to greatly increase the number of stem cells in a culture and, second, they suggest it may be possible to induce stem cell formation with HIF proteins alone or in combination with other stimulating factors without inserting genes at the start of the reprogramming process.

But the findings may also have important implications for cancer research Ruohola-Baker added: Both HIF1α and 2α are known to play an important role in the process in which normal cells are transformed into cancer stem cells from which tumors grow, indeed, the presence of activated HIF1α is known to be a marker for aggressive disease.

The finding of this study suggest, Ruohola-Baker said, that it might be possible to interfere with cancer development by either blocking the effect of HIF1α in malignant cells early in the process or stimulating the effect of HIF2 at a later stage.

More information: Mathieu et al. Hypoxia-Inducible Factors Have Distinct and Stage-Specific Roles during Reprogramming of Human Cells to Pluripotency. *Cell Stem Cell* (2014), dx.doi.org/10.1016/j.stem.2014.02.012

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