What makes stem cells tick?

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Investigators at the Burnham Institute for Medical Research (Burnham) and The Scripps Research Institute (TSRI) have made the first comparative, large-scale phosphoproteomic analysis of human embryonic stem cells (hESCs) and their differentiated derivatives. The data may help stem cell researchers understand the mechanisms that determine whether stem cells divide or differentiate, what types of cells they become and how to control those complex mechanisms to facilitate development of new therapies. The study was published in the August 6 issue of the journal *Cell Stem Cell*.

Protein phosphorylation, the biochemical process that modifies protein activities by adding a phosphate molecule, is central to cell signaling. Using sophisticated phosphoproteomic analyses, the team of Sheng Ding, Ph.D., associate professor at TSRI, Evan Y. Snyder, M.D., Ph.D., professor and director of Burnham's Stem Cell and Regenerative Biology program, and Laurence M. Brill, Ph.D., senior scientist at Burnham's Proteomics Facility, catalogued 2,546 phosphorylation sites on 1,602 phosphoproteins. Prior to this research, protein phosphorylation in hESCs was poorly understood. Identification of these phosphorylation sites provides insights into known and novel hESC signaling pathways and highlights signaling mechanisms that influence self-renewal and differentiation.

"This research will be a big boost for stem cell scientists," said Dr. Brill. "The protein phosphorylation sites identified in this study are freely available to the broader research community, and researchers can use these data to study the cells in greater depth and determine how

phosphorylation events determine a cell's fate."

The team performed large-scale, phosphoproteomic analyses of hESCs and their differentiated derivatives using multi-dimensional liquid chromatography and tandem mass spectrometry. The researchers then used the phosphoproteomic data as a predictive tool to target a sample of the signaling pathways that were revealed by the phosphorylated proteins in hESCs, with follow-up experiments to confirm the relevance of these phosphoproteins and pathways to the cells. The study showed that many transcription regulators such as epigenetic and transcription factors, as well as a large number of kinases are phosphorylated in hESCs, suggesting that these proteins may play a key role in determining stem cell fate. Proteins in the JNK signaling pathway were also found to be phosphorylated in undifferentiated hESCs, which suggested that inhibition of JNK signaling may lead to differentiation, a result that was confirmed in hESC cultures.

These methods were extremely useful to discover novel proteins relevant to the human embryonic stem cells. For example, the team found that phosphoproteins in receptor tyrosine kinase (RTK) signaling pathways were numerous in undifferentiated hESCs. Follow-up studies used this unexpected finding to show that multiple RTKs can support hESCs in their undifferentiated state.

This research shows that phosphoproteomic data can be a powerful tool to broaden understanding of hESCs and how their ultimate fate is determined. With this knowledge, stem cell researchers may be able to develop more focused methods to control hESC differentiation and move closer to clinical therapies.

Source: Burnham Institute (news : web)