New method for creating inducible stem cells is remarkably efficient

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Some of the most challenging obstacles limiting the reprogramming of mature human cells into stem cells may not seem quite as daunting in the near future. Two independent research papers, published by Cell Press in the September 11th issue of the journal *Cell Stem Cell*, describe new tools that provide invaluable platforms for elucidating the molecular, genetic, and biochemical mechanisms associated with reprogramming. The new findings also offer considerable hope toward making the reprogramming process more therapeutically relevant.

Although scientists have successfully reprogrammed mature human skin cells into induced pluripotent stem (iPS) cells by expressing a few key transcription factors, the conversion has been extremely inefficient. "Little is known about the mechanisms by which reprogramming occurs, in part because of the low efficiency," says senior study author Dr. Konrad Hochedlinger from the Harvard Stem Cell Institute. In addition, the iPS cells created thus far have been generated with retroviruses and noninducible lentiviruses, both of which have major limitations that are not compatible with clinical applications.

The Hochedlinger group created a drug-inducible viral system to generate human iPS cells that were molecularly and functionally similar to human embryonic stem cells. This method was unique in that it allowed the researchers to create iPS cells by using the drug doxycycline to control expression of the necessary factors that had been delivered to the cells with viruses.

The researchers then found that when doxycycline was removed and these "primary" iPS cells differentiated to mature cells, another exposure to the drug reactivated the genes required for reprogramming and induced generation of "secondary" iPS cells at a frequency that was far greater that the initial "primary" conversion. The idea of generating these secondary cells was conceived in previous experiments with mice performed in the lab of Dr. Rudolf Jaenisch from the Massachusetts Institute of Technology.

"The secondary system will enable chemical and genetic screening efforts to identify key molecular constituents of reprogramming, as well as important obstacles in this process, and will ultimately lend itself as a powerful tool in the development and optimization methods to produce human iPS cells," explains Dr. Hochedlinger.

In a separate paper, Dr. Jaenisch's group reports on their success in deriving human secondary iPS cells using doxycycline-inducible transgenes. "The drug-inducible system we describe represents a novel, predictable, and highly reproducible platform to study the kinetics of iPS cell generation," says Dr. Jaenisch. "Further, the genetic homogeneity of secondary cells makes chemical and genetic screening approaches to enhance reprogramming efficiency or to replace any of the original reprogramming factors feasible."

Both research teams found that generation of secondary human iPS cells required less time than the initial reprogramming. Interestingly, the time required to generate iPS cells varied among the types of skin cells that were used. For instance, human fibroblasts required several weeks, while keratinocytes required only about 10 days. "The fast kinetics of reprogramming observed for keratinocytes suggests that these cells would be useful for development and optimization of methods to reprogram cells by transient delivery of factors," suggests Dr. Hochedlinger.

The combined results from both research groups represent a major advance toward more efficient strategies for reprogramming differentiated human cells into iPS cells. The methods described here will not only provide critical insight into the reprogramming process, but also, because of the abbreviated time frame, may lead to the generation of cells that will be amenable for therapies, as reprogramming might be achievable without the prohibitive viruses or genetic modifications.

Source: Cell Press