"Generation of Induced Pluripotent Stem Cells without Myc from Mouse and Human Fibroblasts" (2007), by Masato Nakagawa et al. [1]

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In November 2007, Masato Nakagawa, along with a number of other researchers including Kazutoshi Takahashi [6], Keisuke Okita, and Shinya Yamanaka [7], published "Generation of Induced Pluripotent Stem Cells without Myc from Mouse and Human Fibroblasts" (abbreviated "Generation") in Nature [publishedIn]. In "Generation," the authors point to dedifferentiation of somatic cells as an avenue for generating pluripotent stem cells [8] [isBackgroundTo] useful for treating specific patients and diseases. They provide background to their research by observing that previous attempts to reprogram somatic cells to a state of greater differentiability with retroviral factors [9] Oct3/4, Sox2, c-Myc, and Klf4 had succeeded in producing induced pluripotent stem (iPS) cells that contributed to viable [10] adult chimeras [11] and possessed germ line competency [hasRelevance]. However, as they note, the c-Myc retrovirus [12] contributes to tumors in generated chimeras [11], rendering iPS cells produced with c-Myc useless for clinical applications. The authors attempt to overcome this problem by modifying the standard protocol for producing iPS cells in mice in such a way that the c-Myc retrovirus [12] is removed. They identify problems and benefits associated with this method, but most importantly note that their method generated iPS cells that did not cause tumors in chimeric mice. Nakagawa and colleagues also report that they successfully reprogrammed adult dermal fibroblasts to return to a pluripotent state without c-Myc.

The writers open the discussion of their experiment by describing the beginning of their investigation, which originally was aimed at determining whether other family proteins of the four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4, could be used to induce mouse embryonic fibroblasts (MEFs) to return to a pluripotent state. The authors tested Oct1, Oct6, Sox1, Sox3, Sox7, Sox15, Sox17, Sox18, Klf1, Klf2, Klf5, N-Myc, and L-Myc. They discovered that some of these family proteins could successfully reprogram MEFs to become iPS cells, but some could not.

To test whether these proteins cause the MEFs to dedifferentiate to a pluripotent state the experimenters used the Nanog GFP-IRES-Puro′ cassette described in a paper published by Keisuke Okita, Tomoko Ichisaka, and Shinya Yamanaka [7], "Generation of Germline-Competent Induced Pluripotent Stem Cells" [isBackgroundTo] (abbreviated "Germline-Competent"). This gene construct allowed Nakagawa’s group to test for green fluorescent protein (GFP) expression. GFP expression would imply that the cell was also expressing the Nanog gene. Researchers took Nango expression as a clear sign that the cell had dedifferentiated to a pluripotent state.

As the authors of "Germline-Competent" reported in the previous year, no colonies of MEFs tested positive for GFP expression without modification by the c-Myc retrovirus [12]. In "Germline-Competent" Okita et al. report that selection for puromycin resistance (connected with the Nanog GFP-IRES-Puro′ cassette to Nanog expression) began just seven days after exposure to retroviral factors [9]. Nakagawa’s team confirmed that no GFP expressing colonies were obtained after exposing MEFs to only Oct3/4, Sox2, and Klf4, and selecting for puromycin resistance after seven days. However, they found that they could obtain GFP+ colonies without c-Myc when they began selecting for puromycin resistance fourteen or twenty-one days after exposing MEFs to the other three retroviruses. The authors tested the viability [15] of these cells generated without c-Myc by injecting them into mouse [16] blastocysts, which gave rise to adult chimeras [11], confirming that Nakagawa and co-workers had successfully generated iPS cells.

The experimenters also described the results of an experiment done to see whether high-quality iPS cells could be generated without a Myc retrovirus [12] using a method that selected for Fbxo15 expression instead of Nanog expression. Previous experiments had shown that iPS cells obtained using the four standard retroviral factors [9] and the method selecting for Fbxo15 expression differed from embryonic stem (ES) cells in gene expression and in viability [16] for producing adult chimeras [11]. However, as the paper reports, iPS cells produced without the use of a Myc retrovirus [12] via selection for Fbxo15 express ES cell marker genes [17] on levels similar to actual ES cells. Furthermore, these iPS cells are able to contribute to adult chimeras [11]. This is significant because it shows that omitting the Myc retrovirus [12] actually raises the quality of the obtained iPS cells.

Having generated iPS cells from MEFs, both by selecting for Nanog and Fbxo15 expression, and in each case with and without Myc, the authors tested the Myc− iPS cells to see if they would cause tumors in chimeras [11]. They reported that six of the thirty-
Sources

1. Nakagawa, Masato, Michiyo Koyanagi, Koji Tanabe, Kuztoshi Takahashi, Tomoko Ichisaka, Takashi Aoi, Keisuke Okita, Yuji Mochiduki, Nanako Takizawa, and Shinya Yamanaka [7], "Generation of Induced Pluripotent Stem Cells without Myc..."
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