How does C/EBPα speed up cell reprogramming?

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Much of what is known about how mammalian cells decide what to become derives from the study of transcription factor (TF)-induced transdifferentiation and reprogramming into induced pluripotent stem (iPS) cells. Among the plethora of transdifferentiation systems described the C/EBPα-induced conversion of lymphoid progenitors as well as of mature B cells into macrophages is probably the most efficient, reaching 100%.[2] This lineage switch does not involve an overt retrodifferentiation into embryonic stem cells (ESCs) or haemato poetic stem cells, but the transient activation of genes specific for granulocyte/macrophage precursors (GMPs)[3] (and see below).

In contrast to transdifferentiation, cell reprogramming by the Yamanaka TFs Oct4, Sox2, Klf4 and Myc (OSKM) is highly inefficient, as typically only a small percentage of the target cells convert into iPS cells. B cells were found to be particularly resistant to the effects of OSKM, but co-expression of C/EBPα increased the reprogramming efficiency 10–15 fold, such as that ~1% of the cell became reprogrammed.[3] Speculating that the role of C/EBPα is to facilitate the accessibility of chromatin to the Yamanaka factors, we tested whether pre-exposure of B cells to C/EBPα is more efficient than its co-expression. Indeed, we found that a pulse of 18 hours before activation of OSKM increases the reprogramming efficiency of these ‘B+’ cells ca. 100 fold, generating >95% reprogrammed cells within 3 to 4 d.[4] Similar, but weaker effects were observed with human B cells. After OSKM activation B+ cells upregulate endogenous Oct4 as early as after 1 day, followed by Nanog and and Sox2, and reach expression levels comparable to ESCs within a week. Surprisingly, the effect observed was found to be unique to C/EBPα (and its close relative C/EBPβ) and restricted to lymphoid cells, aiding in identifying the immediate substrates modified by the factor.[4]

After a further improvement of our 2-step reprogramming system we have recently conducted an extensive molecular analysis of the reprogramming process.[5] We found that C/EBPα directly binds to and activates enhancers of the key pluripotency TF Klf4 and the DNA modifying enzyme Tet2, and that the latter is required for efficient reprogramming. Surprisingly, a proteomic analysis also revealed that C/EBPα induces a posttranscriptional increase of several hundred proteins, including the histone demethylase Lsd1, found to be required for the silencing of B cell super-enhancers, and the bromodomain protein Brd4, found to be essential for the activation of the pluripotency program. Analyzing the changes in gene expression and chromatin accessibility induced by the C/EBPα pulse revealed that B+ cells resemble GMPs. Of note, GMPs strictly require C/EBPα for their formation and have been reported to be the most susceptible haematopoietic cell type among 10 tested, including HSCs, thus representing naturally occurring ‘elite’ cells.[6] Intriguingly, GMPs are also obligate target cells for oncogene induced acute myeloid leukemia.[7] C/EBPα facilitates the activation of lymphoid progenitors as well as of mature B cells into macrophages, while a C/EBPα pulse followed by OSKM activation results in greatly enhanced formation of iPS cells. Bottom: bars depict rapid downregulation of B cell program, upregulation of pluripotency genes (with key TFs such as endogenous Oct4 and Nanog becoming activated in 2 waves), and post-transcriptional activation of chromatin related factors important for reprogramming.
of other pluripotency factor genes, including Oct4, remains unclear as its regulatory regions are not bound by C/EBPα. A summary of our findings is shown in Figure 1.

Our work raises a number of issues: Is the reprogramming induced by C/EBPα and the OSKM factors a deterministic process? To address this question we are studying the changes in gene expression at the single cell level at various time points of reprogramming. Further, does C/EBPα change the 3D genome organization of B cells, and if so, does this facilitate the subsequent action of OSKM? As we showed that C/EBPα causes looping at the Klf4 enhancer, it is possible that this causes a change in long-range chromatin interactions, a question that we are currently studying using 3C technology. Finally, how does Tet2 influence DNA hydroxymethylation and methylation, and what is its relationship to changes in genome structure? We are addressing these questions by genome wide BS and OxBS sequencing strategies in cells at various stages of reprogramming.

More broadly our work raises the question as to why C/EBPα is so unique in facilitating the formation of pluripotent cells? It is intriguing to speculate that the factor has an as yet undiscovered role in early embryonic development, preceding the formation of the first pluripotent cells. If so, insights gained from reprogramming experiments could help elucidate how mammalian cells make their first decisions during life.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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