Proceedings of ICBioMed 2026 Symposium: Biomedical Engineering and Smart Medicine
DOI: 10.54254/2753-8818/2026.HZ37011

Re-evaluating the Functional Role of Oct-4 in Somatic Cell Reprogramming

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Abstract. This work re-examines the role of Oct-4 in somatic cell reprogramming, particularly during the maturation phase, and challenges the traditional view of Oct-4 as an indispensable initiating factor for pluripotency induction. Although Oct-4 has long been considered a key component of the OSKM system because of its ability to activate pluripotency networks, recent studies by Velychko et al. showed that the SKM combination (Sox2, Klf4, and c-Myc) can generate iPSCs without exogenous Oct-4. These SKM-derived iPSCs exhibit comparable pluripotency characteristics and improved developmental potential, suggesting that Oct-4 is not absolutely required for reprogramming initiation. This analysis highlights that Oct-4 has stage-dependent functions during reprogramming. Rather than acting as the sole trigger of pluripotency, Oct-4 appears to function mainly as a regulatory factor that stabilizes and reinforces the pluripotency transcriptional network during later stages. Prolonged expression of exogenous Oct-4, however, may cause unwanted effects, including abnormal chromatin activation, inappropriate gene expression, and epigenetic disturbances, which can compromise iPSC quality. Evidence from tetraploid complementation experiments further supports this model, showing that SKM-derived iPSCs possess

Keywords: Totipotency, Pluripotency, Induced Pluripoten Stem Cells, Oct-4

1. Introduction

In 2006, Yamanaka and his colleagues reported that introducing four transcription factors: Oct4, Sox2, Klf4, and cMyc (OSKM)—could convert somatic cells into induced pluripotent stem cells (iPSCs). After that, researchers gradually treated Oct4 as the core element driving the whole reprogramming process. Many experimental results showed that Oct4 not only maintains pluripotency in embryonic stem cells (ESCs), but also directly takes part in inner cell mass formation and lineage specification during early embryonic development. Within the traditional reprogramming framework, Oct4 activates the pluripotency transcription network and coordinates downstream gene expression, so people often referred to it as the "master regulator". Even when scientists tried to replace Oct4 with other transcription factors, most successful systems still relied on activating endogenous Oct4. As time went on, this repeated observation made many believe that Oct4 was indispensable.

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As more detailed studies began to explore the molecular mechanisms, this once widely accepted view started to show cracks. In 2019, Velychko et al. [1] published a study in Cell Stem Cell showing that in mouse models, Sox2, Klf4, and cMyc (SKM) alone were enough to drive somatic cells into a pluripotent state without adding exogenous Oct4. Notably, the iPSCs generated by SKM did not exhibit reduced functionality. In tetraploid comple-mentation assays, these cells actually displayed stronger developmental capacity compared with iPSCs derived from the conventional OSKM combination, and they produced fully iPSC-derived mice at a higher rate. This evidence calls into question the long-standing assumption that Oct4 has to be introduced from outside the cell. It also suggests that excessive exogenous Oct4 might disrupt endogenous transcriptional control and epigenetic equilibrium, which could in turn influence the overall quality and diferentiation potential of iPSCs.

As research on totipotency induction continues to deepen, scientists are paying increasing attention to transcription factor dosage, the timing of expression, and the way these factors reshape chromatin structure during reprogramming. To obtain a totipotent-like state, cells must undergo accurate epigenetic reprogramming instead of merely activating pluripotency-associated genes. Viewed from this perspective, Oct4 does not play a simple or fixed role. Its function likely varies with context, and both its expression level and tem- poral dynamics can subtly shape developmental competence. This makes it necessary to reassess how much Oct4 truly contributes to somatic cell reprogramming, so we can more clearly understand how cells transition between pluripotency and totipotency. Such recon- sideration also provides practical insight for improving strategies aimed at generating stem cells with enhanced developmental potential. Based on the findings of Velychko et al., this article revisits the role of Oct4 within the reprogramming network and further examines what these results imply for stem cell developmental potential and totipotency research.

2. Revisiting the role of Oct4: from reprogramming initiation to developmental competence

2.1. The role of Oct-4 in the initiation of reprogramming

Early studies showed that only cmyc could be deleted in the OSKM combination and still maintain a certain reprogramming efficiency, while Oct4 could not be replaced by homologous POU family members [2, 3]. In addition, most of the so-called "Oct4 replacement factors" ultimately achieve pluripotency establishment by activating endogenous Oct4 expression [4, 5], further strengthening the traditional cognition of Oct4 as a reprogramming initiation switch.

However, velychko et al. found that Sox2, KLF4, and cmyc (SKM) could induce mouse fibroblasts to form IPSC clones in the absence of exogenous Oct4 through the systematic transformation of polycistronic vectors. Although the efficiency of SKM system is about 30% of OSKM and the reprogramming kinetics is delayed by about 2 days, the resulting cells show typical ESC characteristics in morphology, expression of pluripotent markers and DNA methylation remodeling. More importantly, this study revealed that co- expression of Sox2 and cmyc can rapidly activate retroviral silencing mechanism in the early stage of reprogramming, which explains why early experiments based on MMLV retroviral system failed to detect reprogramming under Oct4 free conditions [6]. This technical deviation may mask the biological fact that Oct4 is "not absolutely necessary" to some extent.

From the perspective of molecular mechanism, the initiation of endogenous pluripotency network in SKM system is not completely dependent on Oct4. This phenomenon is consistent with the key role of Nanog in stabilizing the pluripotency state at the late stage of reprogramming [7], and also echoes the report that NR5A2, Sall4 and other factors can partially replace Oct4 function [4].

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Chronis et al (2017) found a large number of "non ESC specific" binding sites for Oct4 at the early stage of reprogramming through time-resolved chip SEQ, suggesting that exogenous Oct4 overexpression may lead to abnormal chromatin opening and non target gene activation. In contrast, SKM induction pathway showed a more direct trajectory toward ESC state in principal component analysis, while OSKM system showed a significant deviation in the middle stage [8, 9].

In addition, Shu et al proposed the "seesaw model", which believed that Oct4 and Sox2 promote diferent germ layer programs respectively, and the success of reprogramming depends on the balance of the two [10]. However, the data of velychko et al suggested that SKM did not significantly favor ectodermal fate, while Oct4 overexpression instead induced transient abnormal activation of epidermal related genes. This suggests that the role of Oct4 in in vitro reprogramming may be more embodied as a transcriptional amplifier than a regulator of lineage balance.

To sum up, the existing evidence supports the view that Oct4 is not the only entrance to pluripotency initiation, but an important node for the stabilization and later mainte- nance of pluripotent networks. This repositioning provides a new theoretical basis for understanding the relationship between pluripotency and higher developmental potential states.

2.2. Tetraploid compensation experiments reveal essential differences in reprogramming quality

Although the reprogramming efficiency of SKM system is lower than that of classical OSKM system, velychko et al. [1]. Critically evaluated the developmental potential of the obtained iPSCs through the tetraploid compensation assay, and the results showed that the developmental ability of SKM-induced iPSCs was significantly better than that of OSKM-derived cells. In this experiment, tetraploid embryos can only form extraembryonic tissues, and the fetal body is entirely derived from the injected iPSCs, so this method is considered to be the most stringent standard for testing pluripotency [11]. More importantly, some OSKM-derived full IPSC mice, even if formed, are difficult to survive for a long time, while SKM derived individuals show higher postnatal survival and reproductive capacity.

This phenomenon is not reported in isolation. Previous studies have pointed out that iPSCs are generally weaker than ESCs in terms of developmental potential, and the reasons include loss of imprinting (LOI), epigenetic residual memory and mitochondrial abnormal- ities [12, 13]. For example, aberrant methylation of imprinted gene regions such as zrsr1 and Gtl2 has been shown to be closely associated with tetraploid compensation failure [14, 15]. Velychko et al's study further found that in many high-quality and low-quality IPSC lines examined, 4N of OSKM iPSCs were often accompanied by abnormal methylation of imprinted regions, while SKM derived cells maintained normal imprinting status in most cases. This suggests that the sustained high expression of exogenous Oct4 during reprogramming may interfere with the epigenetic remodeling of imprinted regions.

Transcriptome analysis further supports this notion. . These aberrantly activated genes are rich in target genes repressed by PRC2 complex (such as Suz12) in ESCs [16], indicating that Oct4 overexpression may lead to the imbalance of Polycomb complex regulation, thereby affecting the subsequent differentiation program. Similarly, Chronis et al. [17] found that exogenous Oct4 could bind a large number of non ESC specific sites at the early reprogramming stage, suggesting that it may trigger extensive chromatin remodeling at non physiological doses. It is worth noting that reprogramming efficiency is not simply positively correlated with developmental potential. The traditional view often takes the number of clones as the evaluation index, but velychko et al.'s research emphasizes the concept of "quality first", that is, fewer but closer to the ESC state cell

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population, which may have more application value than the system with high efficiency but accompanied by epigenetic bias.

This is particularly important for the study of totipotency induction. The establishment of totipotency requires not only the activation of pluripotency gene network, but also a comprehensive and accurate epigenetic reset. Therefore, the high developmental potential of SKM system suggests that reducing the interference of non physiological strong drivers in the reprogramming process may be more conducive to obtain high-quality stem cells close to the early embryonic state. This transformation provides important enlightenment for further understanding the boundary between pluripotency and higher potential state.

3. Redefinition of Oct4 function: from promoter to transcriptional amplification and stabilization regulatory node

Based on the existing research evidence, it is necessary to redefine the role of Oct4 in somatic cell reprogramming from an "absolutely necessary priming factor" to a "stable and amplified regulatory node of pluripotency network". The traditional model believes that Oct4 is the core switch of the pluripotent transcriptional network, and its expression is regarded as a prerequisite for successful reprogramming [1, 3]. However, velychko et al. proved through SKM system that in the absence of exogenous Oct4, the endogenous pluripotency network can still be activated, and key factors such as Nanog and Sall4 can be activated at the early stage of reprogramming.

At the same time, a large number of studies have shown that Oct4 has extensive chro- matin binding ability at nonphysiological expression levels. Chronis et al., found that exogenous Oct4 could bind a large number of non-ESC specific sites at the early stage of reprogramming, inducing abnormal enhancer activation [17]. Velychko et al further proved that the OSKM system showed transient activation of a large number of epidermal and lineage-related genes in the middle of reprogramming, and these genes are usually inhibited by the PRC2 complex (such as Suz12) in ESC. It is speculated that exogenous Oct4 may act as a "transcriptional amplifier" in the context of high expression, breaking the original silencing state through nonspecific chromatin opening, promoting pluripotent gene expression in the short term, but increasing the risk of epigenetic perturbation at the same time.

This dual action model can explain why OSKM system has high reprogramming efficiency, but it is not good at developmental potential and imprinting stability. The physiological function of Oct4 is regulated by fine dosage and timing during embryonic development, and minor changes in its expression level can affect lineage selection [18]. Therefore, continuous high-level expression of Oct4 during in vitro reprogramming may break through its normal regulatory range, resulting in excessive chromatin state opening or abnormal activation of lineage genes.

From a broader perspective, Oct4 may not be the "starting point" for the establishment of pluripotency, but the "balancer" for the stable maintenance of pluripotency. Its role is closer to enhancing and stabilizing the existing pluripotency network than the initial trigger signal. This functional redefinition not only corrects the long-standing overemphasis on the central position of Oct4, but also suggests that more attention should be paid to the fine control of transcription factor expression dose and timing when optimizing reprogramming strategies. For the research of pursuing higher developmental potential and even the induction of totipotent state, reducing chromatin perturbation caused by non physiological strong drivers may be more critical than simply increasing the intensity of transcriptional activation.

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4. Conclusion

In order to give a conclusion, it is required for people to give a more clam and rationally thinking about how Oct-4 plays its role in the reprogramming process. Oct-4 has been considered as a switch to trigger the pluripotency for many years. But Velychko et.al. indicated that such claims might be too simple and one-sided. SKM system established a fully functioning pluripotency network without exogenous Oct-4, more than that, it even gives an iPSC with higher potential to be further processed, which is important enough. It suggests that the establishment of pluripotency may not be a linear process triggered by a single core factor, but rather a dynamic network that is gradually reconstructed in stages and layers.

What is more worth to look at is, the exogenous Oct-4 expression somehow comes with a "cost". Although it raises the efficiency of reprogramming, but it can also introduce some implicit epigenetic biases via non specific chromatin binding, abnormal enhancer activation, and interference with the polycomb regulatory network. These biases usually not being detected or not that obvious in pluripotent tests, instead, they are noticed in tetraploid complementary assay or later differentiation.

From the perspective of totipotency induction, such problem is essential. A sample with a higher developmental potential is not only depending on the expression of key factors, but also complete resetting of chromatin structure and clearance of epigenetic memory. Such process may disrupted if the exogenous factors driving too much. Generally, the contribute of Oct-4 is affirmed. It plays a more important role in sta- blizing the pluripotency network rather than initiative factor that unreplacable. The new understanding to this key point is important to both iPSC technique and providing a new pathway of thinking to higher developmental potential iPSC in the future.

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