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TRIM28 epigenetic corepressor is indispensable for stable induced pluripotent stem cell formation

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ABSTRACT

Cellular reprogramming proceeds in a stepwise pathway initiated by binding and transcription of pluripotency factors followed by genome-wide epigenetic changes. Priming events, such as erasure of DNA methylation and chromatin remodeling determines the success of pluripotency acquisition later. Therefore, growing efforts are made to understand epigenetic regulatory network that makes reprogramming possible and efficient. Here, we analyze the role of transcriptional corepressor TRIM28, involved in heterochromatin formation, during the process of reprogramming of mouse somatic cells into induced pluripotent stem cells (iPS cells). We demonstrate that *Trim28* knockdown (*Trim28* KD) causes that emerging iPS cells differentiate immediately back into MEFs therefore they fail to yield stable iPS cell colonies. To better comprehend the mechanism of TRIM28 action in reprogramming, we performed a reverse-phase protein array (RPPA) using in excess of 300 different antibodies and compared the proteomic profiles of wild-type and *Trim28* KD cells during reprogramming. We revealed the differences in the dynamics of reprogramming of wild-type and *Trim28* KD cells. Interestingly, proteomic profile of *Trim28* KD cells at the final stage of reprogramming resembled differentiated state rather than maintenance of pluripotency and self-renewal, strongly suggesting spontaneous differentiation of *Trim28* KD cells back to their parental cell type. We also observed that action of TRIM28 in reprogramming is accompanied by differential enrichment of proteins involved in cell cycle, adhesion and stemness. Collectively, these results suggest that regulation of epigenetic modifications coordinated by TRIM28 plays a crucial role in reprogramming process.

1. Introduction

The reprogramming of somatic cells into pluripotent state can be achieved by a set of defined transcriptional regulators such as OCT4, SOX2, KLF4 and c-MYC, known as OSKM factors or Yamanaka factors (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Despite being an invaluable platform for disease modeling and regenerative medicine, technology of iPS cell generation remains still highly inefficient. The efficiency is remarkably higher for starting cells that are less differentiated such as progenitor cells and depends on the factor delivery method used for induction of dedifferentiation. Nonetheless, still only about 1% of starting cell population is capable of resetting the differentiated state and inducing pluripotency (Stadtfeld and Hochedlinger, 2010). Even if cells are equivalently induced by OSKM-factors, reprogramming process is initially accompanied by a complex network of genome-wide epigenetic modifications that often compose a roadblock in iPS cell derivation (Orkin and Hochedlinger, 2011). Epigenetic remodeling involves several changes in DNA structure including DNA methylation, histone modifications (such as acetylation, methylation and phosphorylation) and nucleosome packaging (M. Li et al., 2012; Surani et al., 2007). Chromatin changes including post-translational modifications of histones can be observed immediately after reprogramming induction, whereas DNA demethylation and X-chromosome reactivation occur in the late stage of dedifferentiation (Koche et al., 2011). Histone acetylation generally correlates with gene activation, while histone methylation can be associated with both activation and repression of transcription depending on the specific lysine residue that is methylated (M. Li et al., 2012). These modifications are accompanied by the recruitment of chromatin-modifying complexes, but their role in reprogramming remains to be determined.

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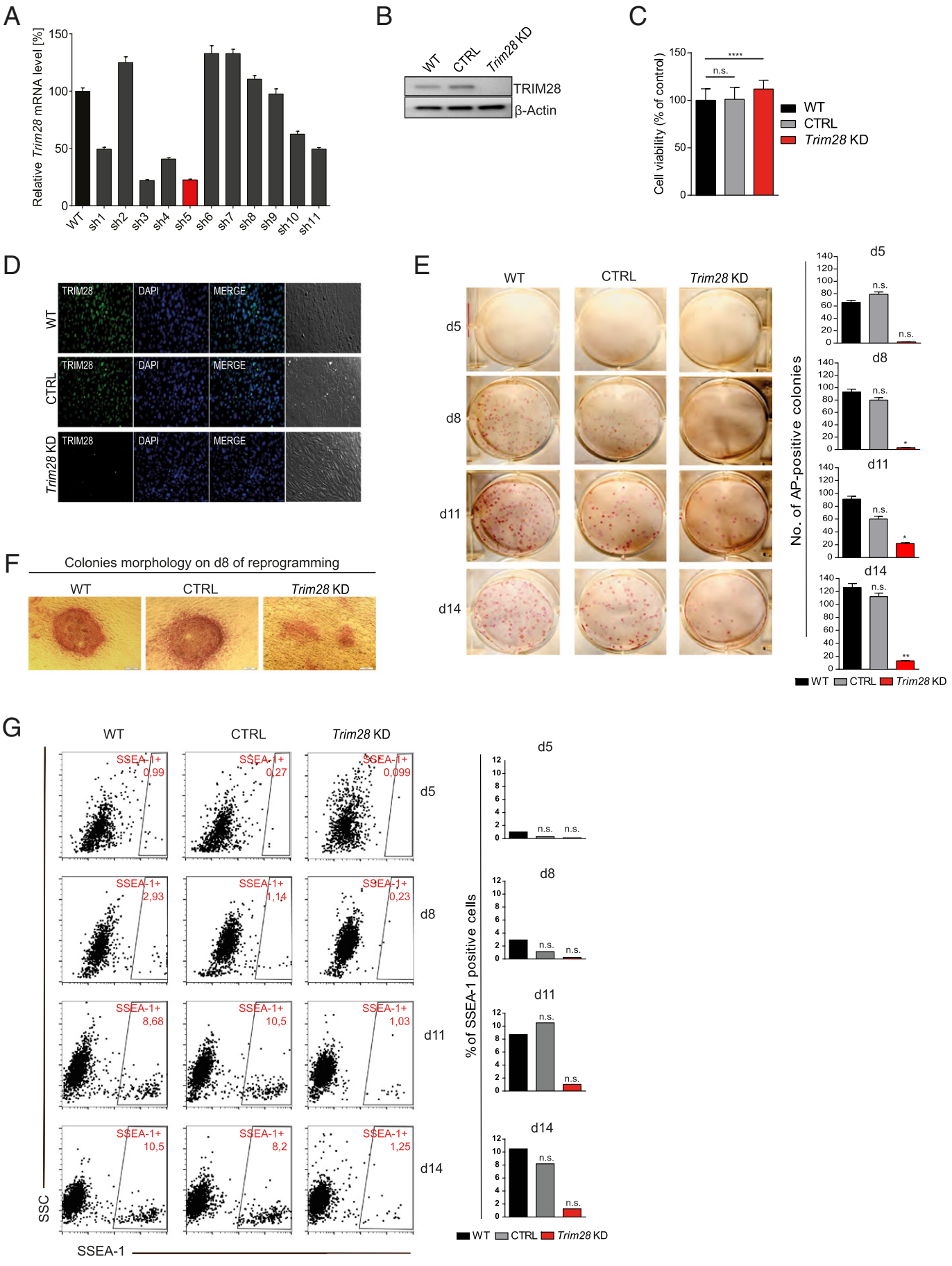
A
Relative Trim28 mRNA level [%]
150
100
50
0
WT sh1 sh2 sh3 sh4 sh5 sh6 sh7 sh8 sh9 sh10 sh11
B
WT CTRL Trim28 KD
TRIM28
β-Actin
C
Cell viability (% of control)
150
100
50
0
****
n.s.
WT
CTRL
Trim28 KD
D
WT
CTRL
Trim28 KD
TRIM28
DAPI
MERGE
E
WT CTRL Trim28 KD
d5
d8
d11
d14
No. of AP-positive colonies
n.s.
*
**
WT CTRL Trim28 KD
F
Colonies morphology on d8 of reprogramming
WT CTRL Trim28 KD
G
WT CTRL Trim28 KD
SSEA-1+ 0,99
SSEA-1+ 0,27
SSEA-1+ 0,099
SSEA-1+ 2,93
SSEA-1+ 1,14
SSEA-1+ 0,23
SSEA-1+ 8,68
SSEA-1+ 10,5
SSEA-1+ 1,03
SSEA-1+ 10,5
SSEA-1+ 8,2
SSEA-1+ 1,25
d5
d8
d11
d14
SSC
SSEA-1
% of SSEA-1 positive cells
n.s.
WT CTRL Trim28 KD