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RESEARCH HIGHLIGHT

"TET-on" pluripotency

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Recent studies have uncovered a specific role of TET proteins in reprogramming somatic cells to induced pluripotent stem cells, a process where O-linked β-N-acetylglucosamine transferase may play a crucial role.

Ten-eleven translocation 1-3 (TET1-3) proteins are DNA hydroxylases that convert the 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in mammalian cells [1]. DNA methylation has been considered one of the major epigenetic modifications involved in X-chromosome inactivation, imprinting, or specifically switching off gene expression, a reversible process where TET proteins have been recently identified as DNA hydroxylases leading to DNA demethylation. Many studies have revealed the crucial role of TET proteins in genome-wide demethylation and gene expression during development and pathogenesis [2]. Here we will focus on the role of TET proteins in reprogramming and pluripotency.

The TET gene was firstly identified as a fusion partner of MLL in acute myeloid leukemia associated with chromosome translocation. In addition to its role in cancer, Rao and colleagues discovered that TET protein possesses enzymatic activity that mediated the conversion of 5mC to 5hmC [3]. The function of TET protein in pluripotency was firstly shown in mouse embryonic stem cells (mESCs) in which TET1 collaborates with DNA methyltransferases to contribute to the maintenance of mESCs, indicating the critical role of TET1 in ESC self-renewal network [1]. In line with these findings, a comprehensive analysis of 5hmC in a genome-wide scale further revealed the significant role of TET proteins in establishing particular pluripotency-associated chromatin contexts [4]. Collectively, these studies demonstrate a link between TET proteins and pluripotency.

In 2006, Yamanaka and colleagues discovered that mouse embryonic and adult fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSCs) by overexpressing four factors Oct3/4, Sox2, Klf4 and c-Myc (OSKM) [5]. NANOG, another pluripotency factor, plays a critical role in maintaining pluripotency, but is dispensable for the initiation of somatic cell reprogramming. NANOG functions as a gateway that induces pre-iPSCs to acquire the ground state of pluripotency. Silencing NANOG causes the inner cell mass to be trapped in a nonviable pre-pluripotent state during development [6]. Many studies have been focusing on the genomic loci occupied and regulated by NANOG. However, little is known about its interacting partners that may contribute to the induction of pluripotency in pre-iPSCs. To identify the interacting partners of NANOG, Costa *et al*. [7] utilized NANOG as bait to identify its physically associated partners via enhanced affinity purification followed by mass spectrometry. TET1 was identified as one of 27 high-confident partners in mESCs. TET1 modulates NANOG function by physical interaction and positively regulates the reprogramming of somatic cells. Silencing TET1 expression led to reduced efficiency in NANOG-facilitated Oct4, Klf4 and c-Myc (OKM)-mediated reprogramming of neural stem cells to pluripotency, whereas ectopic TET1 expression dramatically enhanced its efficiency. TET2 was also found to interact with NANOG. TET-involved reprogramming requires NANOG as overexpression of TET itself had no impact on reprogramming efficiency; whereas overexpression of both TET and NANOG synergized reprogramming efficiency. Co-expression of NANOG and TET1 increased the genome-wide levels of 5hmC. This process is dependent on NANOG-mediated recruitment of TET1 to NANOG-binding sites, suggesting that NANOG cooperates with TET1 in DNA demethylation at specific genomic loci during reprogramming [7]. Interestingly, another study discovered that TET2 and poly (ADP-ribose) polymerase-1 (PARP-1) cooperatively fine-tune epigenetic modifications and upregulate expression of the pluripotency genes *Nanog* and *Esrrb* in the early stage of somatic cell reprogramming [8], indicating a role of TET proteins in the regulation of pluripotency genes' expression as well as their activity. More recently, Gao *et al*. [9] reported that TET1 was able to replace Oct4 to generate fully pluripotent iPSCs, partly by promoting endogenous *Oct4* gene demethylation. Together, these studies indicate that TET proteins may play different roles at different stages of somatic cell reprogramming, ultimately enabling the generation of fully matured iPSCs.

In addition to DNA demethylation, TET proteins can use other strategies to control gene expression. It has been shown that TET proteins associate with either various histone modifications including the methylation of H3K4 and H3K27 or epigenetic regulators such as PRC2 complex and SIN3A co-

repressor complex [2], suggesting that TET proteins could employ multiple mechanisms to regulate chromatin contexts with respect to the control of gene expression. Notably, three groups recently demonstrated that TET proteins can physically interact with O-linked β-N-acetylglucosamine (O-GlcNAc) transferase (OGT) [10-12]. OGT is a unique glycosyltransferase that modifies hundreds of proteins by transferring single O-GlcNAc onto serine/threonine residues, thus participates in signal transduction and transcriptional regulation [11]. Of particular interest, histones are targets of O-linked glycosylation, implying the O-linked glycosylation of histones as a basic epigenetic mark for global or specific gene regulation [13]. The interactions between different TET proteins and OGT are conditional, based on buffer conditions and purification methods. Yu and colleagues used streptavidin-binding peptide (SBP)-tagged TET proteins as bait and identified OGT as an interaction partner of TET2 and TET3, but not TET1 in mESCs. The interactions were further confirmed in 293T cells [10]. Using a flag-tagged biotinylated form of OGT, Pasini's group found that TET1 and TET2 were stable partners of OGT in mESCs [11]. Deplus *et al*. [12] stated that all TET proteins associated with OGT, whereas TET2 and TET3 had a stronger affinity with OGT than TET1 by using Halo-tagged TET proteins followed by affinity purification. Although controversial results were obtained, these studies suggest the following: (1) TET2 interacts with OGT in all three studies. The catalytic domain of TET2 and the tetratricopeptide repeat (TPR) 5 and 6 domains in OGT are essential for the interaction [10]; (2) The interaction between TET proteins and OGT does not affect their enzymatic activity [10]; (3) OGT-TET complex occupies CpG islands at transcriptional start sites (TSSs), where low levels of 5mC and 5hmC but high level of O-GlcNAc-modified histones exist [11, 12]. These data suggest that TET proteins not only function as DNA hydroxylases, but also mediate the recruitment of OGT to genomic loci to modify histones and regulate gene expression [11]; (4) TET proteins recruit OGT and HCF1, an OGT-targeted protein that interacts with the H3K4 methyltransferase complex SET1/COMPASS. Both TET and OGT proteins maintain the integrity of this SET1/COMPASS complex, suggesting a potential novel mechanism of epigenetic regulation (Figure 1) [12].

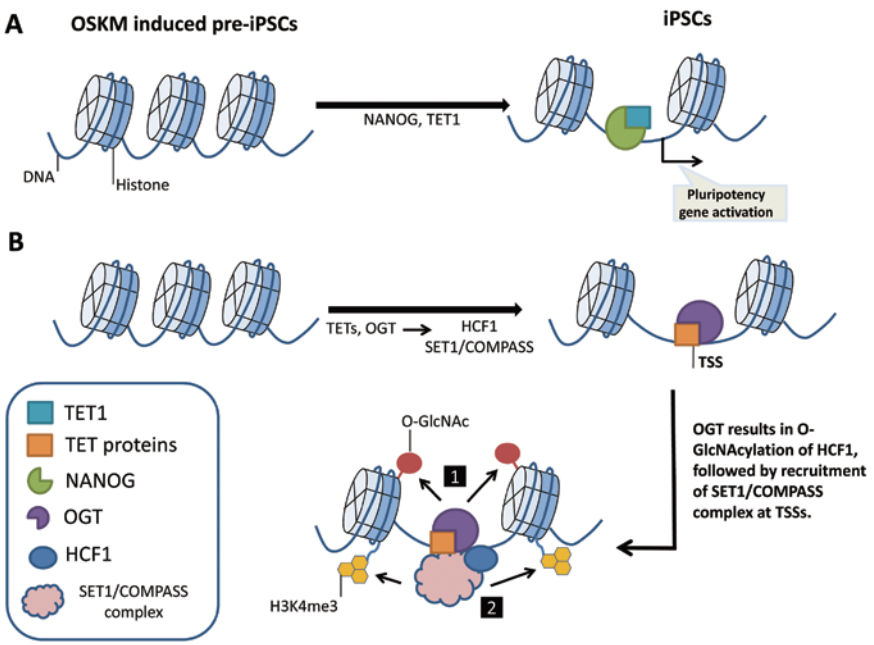

Figure 1 (A) TET1 is recruited by NANOG to demethylate DNA where it occupies and leads to activation of a set of pluripotency genes. **(B)** TET proteins recruit OGT to transcription start site (TSS). In turn, (1) OGT O-GlcNAcylates the adjacent histones; (2) OGT results in O-GlcNAcylation of HCF1, a component of SET1/COMPASS H3K4me3 methyltrasferase complex. The SET1/COMPASS complex modifies adjacent nucleosome by affecting H3K4me3, resulting in transcriptional activation.

Identification of TET proteins is a milestone in the field of epigenetics and resolves a long-standing mystery for DNA demethylation. As TET proteins have the potential to switch on or off specific gene expression, it is not surprising that they have critical roles in reprogramming. The interaction between NANOG and TET proteins has no apparent relevance for the maintenance of pluripotency and self-renewal of ESCs, which renders TET proteins as a promising factor for safe and controllable reprogramming used for regenerative medicine. In addition, TET proteins seem to play multiple roles in gene expression regulation during embryogenesis and early-stage development. In the context of human diseases, as O-GlcNAc is closely related to glucose metabolism in cells, TET proteins may be involved in metabolic diseases such as diabetes through interplay with OGT. TET-OGT interactions may be also involved in aging and cancer progression as genome-wide hypomethylation (in some cases, hypermethylation) of CpG-rich regions has been frequently observed in the aged and transformed human tissues.

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