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Mechanism of DNA Methylation in Stem Cells

Yanning Cai

College of information Science and Technology, Jinan University, China

Abstract

DNA methylation is an important regulation mode in epigenetic mechanism. It affects a series of biological processes by regulating gene expression. As a cell population with self-renewal, high proliferation and multidirectional differentiation potential, the differentiation and regeneration process of stem cells must be accurately regulated. DNA methylation plays an important role in the maintenance, differentiation, and regulation of stem cells. This paper reviews the latest research progress of DNA methylation in embryonic stem cells, pluripotent stem cells and adult stem cells, reviews and prospects the potential biological functions and regulatory mechanisms of DNA methylation in stem cells, and provides some references for clinical diagnosis, treatment, and drug development in the future.

Keywords

Methylation, Demethylation, Stem cells, TET

Epigenetics is proposed by Conrad Waddington about 80 years ago to link genetics and developmental biology. At present, the definition of epigenetics is to study genetic changes in gene expression and function without altering DNA sequences [1]. Epigenetic regulation mainly includes DNA methylation modification (such as cytosine methylation, hydroxy methylation, and recently rediscovered adenine methylation), histone post-translational modification (such as methylation, ubiquitination, acetylation, and phosphorylation) and non-coding RNA-mediated pathway [1-3]. 5-methylcytosine (5 mC) is the most important DNA methylation modification in vertebrates. It plays a key role in regulating gene expression, chromatin structure, gene imprinting, X chromosome inactivation and genome stability [4].

DNA methylation is usually associated with gene suppression. Maintaining proper DNA methylation status is essential for normal development of the body. Therefore, abnormal DNA methylation patterns are associated with the pathogenesis of many diseases, including neurological diseases and cancer [5,6]. In mammals, stem cells are undifferentiated cells that can differentiate into specialized cells and renew themselves through mitosis. Generally speaking, there are two types of stem cells: Embryonic stem cells derived from intra blastocyst mass and adult stem cells existing in complementary or repairing adult tissues. Stem cells can usually be defined by two characteristics: The ability to self-renew over a long period of time and the ability to differentiate into one or more specific cell types. These stem cell characteristics are closely related to epigenetic regulation. Epigenetic modifications, such as DNA methylation, histone modification and nuclear weight remodeling, are widely studied as essential epigenetic control mechanisms for embryonic development, sex chromosome silencing, retrotransposons, and gene imprinting suppression. This review will focus on the latest advances in DNA methylation in stem cell research.

Summary of DNA Methylation

DNA methylation modification is a common epigenetic method, in which organisms, stimulated by different endogenous and exogenous factors, use S-adenosyl-L-methionine (SAM) as a methyl donor and transfer methyl groups to specific structures of DNA bases through DNA methyltransferase (DNMT) biocatalysis. 5 mC usually occurs inside the mammalian genome containing high frequency CG dinucleotides. Three members of the DNMT family (DNMT1, DNMT3a and DNMT3b) are responsible for maintaining and generating 5 mC. DNMT1 maintains DNA methylation in cell cycle by replicating existing patterns of semi methylated DNA into its sub chains during DNA replication.

In contrast, DNMT3a and 3b coordinate with different interacting factors (including histone modifiers or transcription inhibitors) to create new methylation gene regions to achieve their specificity, thus playing the role of de novo methyltransferase [7]. DNA methylation can be

***Corresponding author:** Yanning Cai, College of information Science and Technology, Jinan University, Guangzhou, China

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recognized by a series of reader proteins, such as MeCP2 (methyl-CpG-binding protein 2) and MDB1-4 (methyl-CpG-binding Doman protein 1-4) [8,9]. Abnormal expression of these proteins often has serious consequences, such as neurological diseases and cancer. These phenomena reflect the importance of DNA methylation [10,11]. For a long time, 5 mC was considered the only functional DNA modification type. Until 2009, two different teams reported that 5-hydroxymethylcytosine (5 hmC) may play an important role in development and neuronal activity [12,13]. 5 hmC was first found in bacteriophages in 1953.

Although 5 hmC was found in mammalian genome as early as 1972, the mechanism of 5 hmC production was unknown for a long time. Rao and his colleagues demonstrated that TET1 (ten-eleven translocation 1) is a 2-ketoglutarate and Fe2+ dependent enzyme that oxidizes 5 mC to 5 hmC [13]. Subsequent studies showed that TET family proteins (TET1, TET2 and TET3) could further oxidize 5 hmC to 5-formylcytosine (5 fC) and 5-carboxyl cytosine (5 caC) [14-16]. Both 5 fC and 5 caC can be cut by thymine DNA glycosylase (TDG) and trigger subsequent base excision repair (BER), which gives a new understanding of the regulatory pathway of 5 mC dependence [15,17]. The overall level of 5 hmC varies among tissues.

It is highly enriched in brain tissues such as Purkinje neurons and embryonic stem cells (ESCs), which is about 10 times higher than that in other tissues [12,13]. 5 hmC was initially thought to be only an intermediate link in demethylation, but subsequent studies have shown that 5 hmC can be stable in the brain and grow with development and age [18]. Similar to 5 mC, 5 hmC can also be recognized by specific proteins [19] and has certain biological functions [20]. Recently, in the genome of higher eukaryotes, including green algae, nematodes, fruit flies, toads, zebrafish, pigs, and mice, a N6-(N6-methyladenine, m6A) [21-27], which is ubiquitous in prokaryotes, has been discovered. Although these studies indicate that m6A has potential epigenetic effects, its exact biological functions are still in the initial stage of exploration.

The Role of DNA Methylation in Embryonic Stem Cells and Pluripotent Stem Cells

Methylation in embryonic stem cells and reprogramming of iPSCs

CpG methylation in mammals is a specific epigenetic mechanism that promotes the regulation of gene expression. In addition to CpG methylation, methyl can also be added to cytosine which is not upstream of guanine. This form of DNA methylation is called non-CpG methylation and is widely found in plants. Non-CpG methylation has also been reported in mammals, such as embryonic stem cells [28-30]. Dnmt1 is essential for mouse embryonic development, while ESCs of Dnmt1 mutant mice have normal self-renewal, but their differentiation is impaired [31,32]. Dnmt3a and Dnmt3b are necessary for the early development of mice. Targeted blocking of these two genes can block methylation of new genes in embryonic stem cells and early embryos, but usually has no effect on the maintenance of methylation imprinting mode [7].

However, for repetitive sequences containing LINE-1 promoter in mESCs, the maintenance of methylation by DNMT1 alone is insufficient, while DNMT3a and DNMT3b can make up for the deficiency of DNMT1 [33]. DNA methylation of DNMT1 or DNMT3a/b plays an important role in development, but in mice ESCs with triple knockout of Dnmt1 -/- Dnmt3a -/- Dnmt3b -/- even though there is no DNA methylation at all, mice ESCs still have the function of self-renewal [34]. Changes in DNA methylation are essential for the reprogramming of induced pluripotent stem cells (iPSCs) because many pluripotent gene promoters must have methylation deletion, namely demethylation [35]. DNA methylation occurs only at the late stage of iPSC reprogramming. If the methylation of multipotent gene DNA is not removed, cells can only partially reprogram [36]. DNA methylation with epigenetic memory exists in low-pass algebraic mice and human iPSCs, which is conducive to their differentiation into donor cell lines but restricts their differentiation into other cell types [37,38]. 5-azacytidine, a DNMT inhibitor used in the reprogramming process, increased the reprogramming efficiency by about 10 times [39].

These findings suggest that demethylation usually plays an important role in reprogramming, and DNA demethylation is an inefficient step in building pluripotency. Contrary to DNA demethylation, DNA methylation did not contribute significantly to iPSCs reprogramming [40]. Dnmt3a and Dnmt3b are highly expressed in embryonic stem cells and are strongly induced after pluripotency is established. However, initial DNA methylation is not important, and it is not necessary for somatic nuclei to reprogram into pluripotent states [41]. This suggests that somatic gene silencing may be initiated mainly through different mechanisms, such as H3K27 methylation or H3K9 methylation, the important role of PRC2 (polycomb repressive complex 2) and H3K9 methyltransferase in reprogramming is good evidence [42-44].

Role of TET protein and 5 hmC in multipotent stem cell reprogramming

The overall level of 5 hmC is very high in mESCs and human ESCs (hESCs). For example, in mESCs, 5 hmC accounts for 0.04% of all nucleic acids or 5% ~10% of total mC [13]. It is reported that 5 hmC participates in the differentiation process [14,45]. TET1 and TET2 are abundantly expressed in mESCs [46]. TET1 and TET2 seem to have different characteristics in mESCs. For example, the deletion of TET1 can decrease the 5 hmC level of the transcription initiation site, while the deletion of TET2 is mainly related to the decrease of 5 hmC in the gene [47]. The decrease of 5 hmC caused by double knockout of TET1 and TET2 will keep cells pluripotent, but it will lead to developmental defects in chimeric embryos [48]. In addition, the gene expression in undifferentiated hESCs with triple knockouts of TET1, TET2 and TET3 did not decrease correspondingly, but the hypermethylation of bivalent promoters occurred significantly, indicating that TET protects bivalent promoters from hypermethylation, and also limits the normal differentiation of embryonic stem cells [49]. 5 hmC can be further oxidized to 5 fC and 5 caC, both of which can produce unmodified cytosine [15,17,50] through TDG repair. Regulators have been reported to be involved in this

process, linking this mechanism to various cellular functions. For example, in mESCs, TET proteins are the substrates of direct calpains. Calpain1 mediates the stability of TET1 and TET2 after transcription, and calpain2 regulates the level of TET3 during differentiation [51].

In addition to cytosine modification, cells also contain 5-hydroxyme-thyluracil (5 hmU), which is a product of reactive oxygen species. Recent reports have shown that TET-induced oxidation is not limited to 5 mC, and thymine is also a substrate that can be catalyzed to 5 hmU. Therefore, besides triggering DNA repair pathways, 5 hmU may have other functions in cells [52]. Considering that DNA demethylation may be modified by TET-BER process, many research groups have studied whether TET-mediated 5 hmC is involved in reprogramming and how it contributes to this process [53-56]. TET1 and NANOG work together to enhance the efficiency of reprogramming. The co-expression of NANOG and TET1 increased the level of 5 hmC on target gene loci Esrrb and Oct 4 [53].

In another study, TET1 overexpression promotes fibroblasts to form reprogrammed cloned cells, and the authors believe that TET1 accelerates transcriptional activation of Oct 4 through demethylation of its promoter [55]. This model provides important experimental evidence that TET1 can replace Oct 4 to produce fully versatile iPSCs. The reprogramming of TDG-deficient fibroblasts is also affected. The barriers to reprogramming are at least partly due to the activation of defects in some microRNAs, which depend on demethylation promoted by TET and TDG [57]. Similar to many other systems, TET regulation during reprogramming has complex regulatory networks. For example, microRNA-22 is not only a repressor of TET protein, but also a powerful oncogene [58,59] in mammary epithelium and hematopoietic system. MicroRNA-29a/b also participates in the regulation of TET [59]. Because the expression of microRNA-22 and microRNA-29a/b in fibroblasts and iPSCs is different [60], these microRNAs may regulate the expression of TET at post-transcriptional level, thus inhibiting somatic cell reprogramming.

Interestingly, ascorbic acid (vitamin C) regulates TET activity. Some reports have shown that vitamin C increases 5 hmC levels 3-7 times [61-64] in cell DNA, indicating that it is a direct regulator of TET activity and a promoter of DNA demethylation. In terms of mechanism, vitamin C may promote replication-dependent and passive DNA demethylation by enhancing the formation of 5hmC and accelerate active DNA demethylation by enhancing the formation of 5 fC and 5 caC. In iPSCs reprogramming, vitamin C is considered to be able to overcome aging blockade, promote pre-iPSCs conversion [65], and improve H3K36 demethylase activity [66]. These are important factors to improve reprogramming efficiency, which also means that TET1 has a positive impact on reprogramming. However, one study showed that TET1 -/- MEF cells were more efficient when converted from standard iPSCs medium to improved vitamin C-rich medium. This contradictory finding reveals the relationship between vitamin C and TET1 reprogramming. According to the report, some people believe that TET1 can regulate somatic cell reprogramming positively or negatively depending on the presence or absence of vitamin C [64].

Role of DNA Methylation in Neural Stem Cells

Neural stem cells (NSCs) are the main part of the central nervous system (CNS) produced by self-renewal and pluripotent cells. The discovery of adult NSCs is a milestone in understanding the plasticity of the adult brain because it overturns the traditional idea that the central nervous system of adult mammals does not produce new neurons [67,68]. The niche of stem cells in the nervous system is in the endothelial cells located at the base of the subventricular zone and the sub granular zone of the dentate gyrus of the hippocampus [69-71]. Adult NSCs mainly produce neurons and glial cells, including astrocytes and oligodendrocytes [72]. The process of generating functional neurons from adult NSCs is defined as adult neurogenesis [73-75]. Adult neurogenesis can be regarded as a classic case of stem cell differentiation. Genetic regulators subtly manipulate the expression of key genes in neural stem cells in time and space and determine the proliferation and differentiation of neural stem cells.

DNA methylation in adult neural stem cells

DNA methylation plays an important role in synaptic plasticity related to learning and memory by regulating specific gene expression. In order to explore the role of DNA methylation in CNS, many conditional knockout mice models have been developed. DNMT1 is widely expressed in mouse brain, and the elimination of DNMT1 in neuronal progenitor cells leads to DNA hypomethylation, while activating JAK-STAT astrocyte production pathway and accelerating the process of glial differentiation [76,77]. DNMT3a activates cells near E10.5, the precursor of mouse neurons, and maintains activity in adult mitotic neurons [78]. Mice lacking DNMT3a throughout the central nervous system looked healthy but died prematurely from developmental defects [79]. With the loss of DNMT3a, neurodevelopmental disorders appeared in both the subventricular area and the sub granular area of the dentate gyrus of the hippocampus, and the number of neurons differentiated from NSCs with the loss of DNMT3a decreased by 90% [80].

In addition, DNMT3a competes with Polycomb through methylation of non-proximal promoters and promotes transcription of targets including neurogenic genes [80]. MBD1 is a protein that binds to the promoter of hypermethylation gene and is an indispensable regulator of DNA methylation-dependent adult NSCs. Fgf2 (fibroblast growth factor 2) is a mitogen of adult neural progenitor cells. The promoter of Fgf2 hypermethylation can be bound by MBD1. Therefore, the deletion of MBD1 induces hypomethylation of Fgf2 promoter and enhances its expression in adult NSCs, resulting in inhibition of NSCs differentiation. Active DNA methylation/demethylation has been reported to play a key role in neurogenesis.

Gadd45b (growth arrest and DNA damage-inducible protein 45b) is considered to be a key regulator of adult neurogenesis. MeCP2 in mature neurons can interpret reversible DNA methylation under the regulation of DNMTs

and Gadd45b proteins [78,81]. In addition, some microRNAs, such as microRNA-184 and microRNA-137, can be used as direct targets for MBD1 and MeCP2, respectively, to inhibit neuronal differentiation from adult NSCs [82-84]. MeCP2-mediated epigenetic regulation of microRNA-137 also involves co-regulation of the core transcription factor Sox2 of stem cells to regulate the fate of adult NSCs [84].

Role of DNA hydroxy methylation and TET in adult neural stem cells

The specific distribution of 5 hmC in mammalian brain and its role in gene regulation indicate that 5 hmC plays an important role in neuronal development and may play a role in neurological diseases [85]. The results showed that the total amount of 5 hmC in neurons increased significantly from neurodevelopmental stage to adult stage [18]. Given the high level of 5 hmC in the brain and its binding protein has been identified, 5 hmC is considered to be more than just an intermediate for DNA demethylation. TET1-mediated 5 mC/5 hmC conversion showed that 5 hmC was more easily demethylated by AID (activation-induced deaminase)/ APOBEC (apolipoprotein B mRNA editing enzyme complex) [86]. Overexpression of TET1 or AID in dentate gyrus of hippocampus resulted in significant decrease in promoter CpG methylation levels of two neuronal activity-related genes Bdnf and Fgf1b [86].

In conditioned mutant mice, the deletion of TET1 resulted in a 45% decrease in NSCs in the subventricular zone of the lateral ventricle and impaired growth function of neurons isolated from TET1 -/- mice in vitro [87]. Researchers found that many genes involved in NSCs proliferation in TET1 -/- mice were hypermethylated and down-regulated [87]. In addition, the knockout of TET2 promotes the proliferation and inhibits differentiation of adult NSCs. TET2 interacts with Foxo3a (transcription factor for khead box O_3) to regulate the expression of important NSCs genes and plays an important role in transcriptional regulation of neurogenesis [88].

Role of DNA Methylation in Germinal Stem Cells

During the development of implanted mammalian embryos, a small group of posterior proximal epiderm of E6.25 separated from somatic cells and presented the fate of germ cell differentiation. Shortly thereafter, these cells were induced by signals from the embryonic ectoderm and became primordial germ cells (PGCs) on E7.25 [89,90]. From the migration of PGCs to the early stage of gonad (E7.75-E12.5), the epigenetics of the whole genome changed. In this process, PGCs escaped the fate of somatic cells and acquired the developmental potential of germ stem cells (GSCs) [91,93]. Given the gamete nature of generations that produce genetic information, GSCs are also considered to be truly "immortal stem cells" [94].

In model invertebrates, if flies and Caenorhabditis elegans, long-term self-renewal of GSCs exists in both males and females [95]. In mammals, males maintain spermatogenesis of GSCs in the testis [96], while it is still controversial whether females have GSCs after birth [97-99]. Mouse GSCs have been successfully cultured in vitro to restore fertility of testicles depleted by germ cells [100,101]. In vitro cultured mouse GSCs can also produce ESCs-like cells that can differentiate into different cell types [100,102].

DNA methylation and demethylation of adult germ stem cells

Specific high-resolution maps of 5 mC, 5 hmC, histone modification and RNA-seq in adult germ stem cells (AGSCs) and spermatogenesis have been drawn. Studies have shown that there are differences in DNA methylation sites between AGSCs and ESCs during reproductive development. By comparing DNA methylation sites between AGSCs and ESCs, about 330 differentially methylated regions were found. These regions are concentrated on the promoter of ESCs silencing gene. Between PGCs and AGSCs of E16.5, many key genes related to meiosis lose DNA methylation and many key genes responsible for cell migration are silenced. It is worth noting that there is no significant change in DNA methylation during gametogenesis, but the transcription of promoters carrying 5 hmC, low CG and high H3K9ac and H3K4me3 has changed significantly. In addition, enhancers of functional genes such as Nanog, Sox2 and Prdm14 are usually hypomethylated and divalent in mature sperm, suggesting that zygotes may have active DNA demethylation [103].

Role of TET and DNA hydroxy methylation in primordial germ cells

In principle, PGC reprogramming must simultaneously inhibit ongoing somatic cell processes and activate germ cell transcription networks to ensure the success of GSCs singularity, leading to gametogenesis [91,104]. The results showed that 5 mC and 5 hmC were significantly distributed in different regions during reproductive cell reprogramming of wild-type PGCs, indicating that the dynamic changes of DNA methylation in proper germ cell maintenance were very important [105-107]. The demethylation and imprinting of PGCs are always accompanied by the conversion from 5 mC to 5 hmC, which is mainly mediated by TET1 and TET2 [107]. In addition, TET3 was expressed in late gametogenesis (E16.5) and spermatogenesis and oocyte, while the elimination of TET3 in early embryonic mouse oocyte resulted in epigenetic abnormalities in the paternal genome [108].

Conclusions and Prospects

Although the mechanisms of DNA methylation and demethylation in embryonic stem cells, somatic cell reprogramming and adult stem cells have been extensively studied, the detailed regulatory mechanisms and signal transduction involved in DNA methylation and demethylation in stem cells are still limited. Further exploring the exact molecular mechanism of DNA methylation and demethylation, the complex relationship between DNA methylation and other epigenetic modifications is the basis for understanding its regulatory function in stem cell maintenance and differentiation process, which is also a hot topic in the future related fields. Theoretical research and practical application

in this area are of great significance. The goal of regenerative medicine is to create and replace damaged tissues and organs through stem cell therapy, especially the directional induction of pluripotent stem cells, which has always been the difficulty of stem cells and regenerative medicine research.

On the one hand, the effect of DNA methylation on cell differentiation propensity may be helpful for the directional induction of iPSCs in order to design a better therapeutic regimen; on the other hand, the stability of genomic modification should be paid attention to when using stem cells and their derivatives in clinical treatment. It is believed that theoretical research and extensive development and application of new technologies in this field will certainly promote the development of this field and provide alternative treatment strategies for future human transplantation and related degenerative diseases.

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