Epigenetic regulation of mammalian sex determination

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Abstract: Epigenetics is the study of changes in gene function that cannot be explained by changes in DNA sequence. A mammalian body contains more than two-hundred different types of cells, all derived from a single fertilized egg. Epigenetic gene regulation mechanisms essentially contribute to various processes of mammalian development. The essence of epigenetic regulation is the modulation of gene activity through changes in chromatin structure. DNA methylation and histone modifications are the major epigenetic mechanisms. Sex determination is the process of establishing a gender. Sry, the sex-determining gene in therian mammals, initiates testis differentiation. Recent studies have provided evidence that epigenetic mechanisms contribute to Sry regulation.


Keywords: sex determination, DNA methylation, histone modification, polycomb group proteins

1. INTRODUCTION

The regulation of gene expression without changes in the DNA sequence is governed by epigenetic mechanisms. Epigenetic mechanisms contribute to numerous biological processes, not only in higher eukaryotes but also in single cell eukaryotes. For example, epigenetic mechanisms control mating type silencing in yeasts, temperature-dependent vernalization in plants, position-effect variegation in insects, and germ line imprinting and X-chromosome inactivation in mammals. DNA methylation, histone modification, non-coding RNA and chromatin remodeling are the major players in epigenetic regulation (1). Among them, DNA methylation and histone modification have been most extensively studied.

DNA methyltransferases (DNMTs) catalyze methylation at the 5′ carbon of a cytosine (5mC) next to a guanidine (CpG). Mammals have two types of DNMTs. DNMT1 acts as a maintenance methyltransferase that catalyzes the methylation of hemimethylated DNA sequences, while DNMT3 acts as a de novo DNA methyltransferase that catalyzes the methylation of unmethylated DNA sequences (2). Recently, the ten-eleven translocation 1-3 (Tet1-3) proteins have been found to possess DNA hydroxylase activity toward 5mC. The Tet1-3 proteins can convert 5mC into 5-hydroxymethyl cytosine (5hmC), which is considered to be an intermediate in the process of active DNA demethylation (3).

The nucleosome is the fundamental unit of chromatin, and consists of 147 base pairs (bp) of DNA wrapped around a core histone octamer (two each of H3, H4, H2A and H2B) (4). The tail regions of the core histones are susceptible to a variety of covalent modifications, including acetylation, phosphorylation, methylation, and ubiquitination (5). These modifications can be reversed by the corresponding deacetylase, phosphatase, demethylase and deubiquitinase. Distinct combinational sets of histone modifications are considered to regulate unique biological outcomes. This concept is referred to as the “histone code hypothesis” (6).

The structure of the epigenome can be modulated by environmental changes. For example, vernalization in flowering plants requires the methylation of specific histone arginine and lysine residues (7). In animals, the nutrition status during development can lead to locus-specific changes in the epigenome. For instance, methyl donor supplementation of pregnant female mice induces the CpG hypermethylation of a specific allele in their offspring (8). On the other hand, the activities of chromatin modification enzymes are dependent on high-energy metabolites as cosubstrates. DNMTs use S-adenosyl methionine as the methyl donor. The kinases, acetyltansferases and methyltransferases acting on histones require ATP, acetyl-CoA and S-adenosyl methionine as the phosphoryl, acetyl and methyl donors, respectively. Chromatin modification enzymes sensitively monitor environmental and metabolic events, and thus function as sensors of changes in these conditions (9).

In mammals, a single fertilized egg differentiates into more than two-hundred different types of cells during development. Epigenetic mechanisms essentially contribute to this process, by regulating gene expression in spatial and temporal manners. Sex determination is the genetic or environmental process by which the gender (male or female) of an individual is established, in a simple binary fate decision. A gene called sex determining region Y chromosome (Sry) was identified as a candidate for a mammalian sex determining gene (10). The introduction of a genomic fragment containing Sry generated male mice, although they were chromosomally female mice, indicating that Sry is necessary and sufficient for testis induction (11). The SKY protein is the founding member of the SOX (SKY-related HMG box) family of transcription factors. Sry expression is restricted to a subset of gonadal somatic cells from embryonic day (E) 10.5 to E12.5 in mice. This spatial and temporal Sry regulation is critical for testis differentiation (12, 13). In this review, I will particularly focus on the epigenetic regulation of Sry expression.

2. ROLE OF DNA METHYLATION IN MAMMALIAN SEX DETERMINATION

1) DNA methylation profiles of the Sry promoter in developing mice embryos

Sixteen CpG sites exist in the 4.5-kb 5′-flanking region of the mouse Sry locus (14). A sodium bisulfite sequencing analysis revealed that the CpG sequences of the Sry promoter region were hypermethylated in E8.5 embryos, in which Sry was not yet...
expressed. However, this region became hypomethylated specifically in the XY gonad at E11.5, while it was still hypermethylated in the other tissues where Sry was not expressed (14). Thus, an inverse relationship exists between the Sry expression levels and the extent of DNA methylation. H3K9 methylation of promoter region DNA is generally associated with actively transcribed genes. The cause and effect relationship between the expression of Sry and the hypomethylation of its promoter region is currently unclear, and deserves further study.

2) Do Gadd45 family proteins contribute to the DNA demethylation of the Sry locus?

The GADD45A, B, G proteins are a family of stress-response proteins. GADD45 mediates diverse cellular processes, such as DNA repair, apoptosis, cell cycle arrest and senescence (15, 16). The GADD45 proteins also function in gene activation, by promoting DNA demethylation and MAPK signaling. The Gadd45 proteins are considered to recruit DNA repair proteins to specific loci, in order to initiate DNA demethylation (17).

Gadd45g-mutant mice display complete male-to-female sex reversal. Sry expression is reduced in the undifferentiated gonads of Gadd45g-mutant embryos, suggesting that GADD45G positively regulates Sry expression (18, 19). Unexpectedly, a bisulfite sequencing analysis revealed that the CpG sequences within the Sry promoter region of undifferentiated gonadal somatic cells were still hypomethylated in the Gadd45g-mutant embryos. These facts suggested that GADD45G activates Sry expression in a different manner than by the CpG demethylation of Sry. Alternatively, GADD45G activates Sry in a different manner than other CpG demethylation of Sry. GADD45G binds and activates MAP3K4. The activated MAP3K4 sequentially activates p38MAPK, resulting in the direct or indirect activation of GATA4, which is implicated in the regulation of Sry expression (Figure 1) (20).

3. ROLE OF HISTONE METHYLATION IN MAMMALIAN SEX DETERMINATION

Histone methylation was previously considered to be an irreversible modification that could only be removed by histone exchange or dilution during replication. The identification of the Lysine-specific demethylase 1 (LSD1) and Jumonji C (JMJC) histone demethylase enzyme families resulted in a quite different viewpoint of the regulation of histone methylation (21, 22).

The methylation of histone H3 lysine 9 (H3K9) is a hallmark for transcriptionally silenced heterochromatin, and is conserved from fission yeast to mammals (23, 24). Jmjd1a (also called TSGA/JHDM2A/KDM3A), an enzyme that demethylates H3K9, plays an important role in gene activation in spermiogenesis and metabolism (25–28). Recently, Kuroki et al. reported that XY mice deficient in Jmjd1a exhibit male-to-female sex reversal. The development of external and internal genitalia in XY Jmjd1a-mutant mice was variable. Approximately 20% of the XY Jmjd1a-mutant mice had male external genitalia, and the others had ambiguous or female external genitalia (Figure 2). Sry expression is perturbed in Jmjd1a-mutant XY gonads at E11.5 (29). Three different approaches were employed to determine the critical step(s) in the testis-developing pathway controlled by Jmjd1a. First, a microarray analysis revealed that the expression levels of the known positive regulators of Sry were not compromised by the Jmjd1a mutation. Second, a rescue of the sex-reversal phenotype was attempted by experimentally restoring Sry function, by crossing the Hsp-Sry transgenic mouse line (30) into the Jmjd1a-deficient background. Consequently, the forced expression of the Hsp-Sry transgene rescued the sex-reversal phenotype of the Jmjd1a-deficient mice. Finally, a chromatin immunoprecipitation analysis revealed that Jmjd1a accumulates on the Sry locus in undifferentiated XY gonads, and mediates its H3K9 demethylation. Taken together, these results revealed that Jmjd1a specifically contributes to the Sry activation process.
shown on the left and right, respectively. Among 58 XY
development of internal genitalia in XY
ous external genitalia (B) and 33 have female external genitalia (C). The
animals examined, 11 have male external genitalia (A), 14 have ambigu-
mary gland, respectively. Te, testis ; Ov, ovary.

step during the testis-development pathway, by directly catalyzing
H3K9 demethylation (Figure 1) (29).

4. ROLE OF POLYCOMB GROUP PROTEINS IN MAMMALIAN SEX DETERMINATION

The polycomb group (PcG) proteins were identified as molecules
required for maintaining the repressed state of homeotic genes in
Drosophila (31). The functions of the PcG proteins are highly con-
served, from Drosophila to mammals. In vertebrates, the PcG pro-
teins assemble into two distinct complexes, polycomb-repressive
complex 1 (PRC1) and polycomb-repressive complex 2 (PRC2).
The PRC complexes at least partially exert their functions through
chromatin modification, because both of the PRC complexes pos-
sess histone modification activities. PRC1 and PRC2 catalyze the
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Dysregulation of the epigenetic machineries is associated with
several human diseases (41, 42). Disorders of sex development
(DSDs) are congenital conditions, in which chromosomal, gonadal,
and/or anatomical sex is atypical (43). Mammals employ GSD,
where sex is determined at conception due to the genetic differ-
ces of zygotes. However, it seems likely that the epigenetic
machineries also play important roles in the regulation of sex-
determining genes in mammals, as reviewed in this article. In
addition, more than half of the human DSDs cannot be explained
by alterations in the characterized genes required for sex determin-
ation and gonadogenesis (44). Collectively, alterations of the epi-
getic machineries and/or epigenetic states may be responsible
for the onset of DSDs. Accordingly, the CBX2 gene mutation was
found in a human exhibiting a male-to-female sex reversal pheno-
type (45). Given the accumulating studies in the epigenetics re-
search area, many new insights will emerge to reinforce the links
between epigenetic mechanisms, sex determination, and gonado-
genesis.

DISCLOSURE OF CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. Brock HW, Fisher CL: Maintenance of gene expression pat-
terns. Developmental dynamics : an official publication of the
American Association of Anatomists 252 : 633-655, 2005
of mammalian DNA methyltransferases. Cellular and molecu-
lar life sciences : CMLS 61 : 2571-2587, 2004
3. Wu H, Zhang Y: Mechanisms and functions of Tet protein-
mediated 5-methylcytosine oxidation. Genes & development
25 : 2436-2452, 2011


