—Mini Review—

Epigenetic Regulations in Placentation

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Abstract: Epigenetic regulation of gene expression plays critical roles in differentiation of cells and organs. In mammalian placentation, it is clearly shown that genomic imprinting which is primarily controlled by DNA methylation is essential for placentation. Addition to DNA methylation, histone modifications and non-coding RNAs are also involved in placentation. Recently, it has been shown that epigenetic mutations could cause medical complications during pregnancy such as FGR (Fetal Growth Restriction) and PIH (Pregnancy Induced Hypertension). Developmental epigenetics would contribute to establish new concepts of diseases and provide new treatments in future.

Key words: Epigenetics, DNA methylation, Histone modification, Fetal growth

Introduction

Human Genome Project was completed in 2003. It was the national project and took more than 10 years and cost several billions of dollars. Knowledge of the project innovate our methods drastically. Nowadays, you can get a whole genome sequencing results only for 1,000 dollars within approximately two months. What you need to do is just send your genomic DNA to a company. Whole genome sequencing is not a special analysis anymore.

On the other hand, a lot of life phenomenon and diseases still remain unknown and/or unexplained even after human genomic sequences were read. The new progress of research fields in the “Post Genome Sequence Era” is indispensable for further development of the reproductive medicine and biology. Therefore, I focus on topics of epigenetics in this review and introduce the basics of epigenetics participating in placentation and in human diseases.

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Definition of Epigenetics

Though nuclei of approximately 60 trillion cells constituting single human have identical genetic information fundamentally, the cells differentiate into different cells, different organs completely. Besides, dedifferentiation and redifferentiation do not occur after final cell fate, and they strictly maintained themselves. These are a matter of common knowledge, but how are different cells produced by the identical genomic information, and how do cells in organs maintain themselves without maldifferentiation for dozens of years? The total picture remains unknown, but some inheritable information except conventional “genetic information” exists and is transmitted after cell division to maintain their functions. The inheritable information except DNA sequences, that is, “epigenetics” makes cell functions stable for long term.

Epigenetic Mechanisms in Placentation

DNA methylation

Three DNA methyltransferase genes, *DNMT1*, *DNMT3A* and *DNMT3B* are identified and DNMT1 is called a maintenance methyltransferase because the DNMT1 protein has a strong enzyme activity as a hemi-methyltransferase which recognize newly synthesized strand in replication of DNA. DNMT3A and DNMT3B are so called “*de novo* type” DNA methyltransferases and have enzymatic activity to make unmethylated DNA methylated.

In mammalian early development, genome-wide DNA methylation is erased by the blastocyst stage after the fertilization (Fig.1) [1]. However, the methylation of imprinted regions escapes erasure. From embryo implantation till gastrulation stage, strong *de novo* DNA methyltransferase activity is observed. After removal of the DNA methylation, a specific DNA methylation pattern depending on cell fates and specific organ differentiations are established, then specific gene expression patterns are established subsequently. At this period, extraembry-

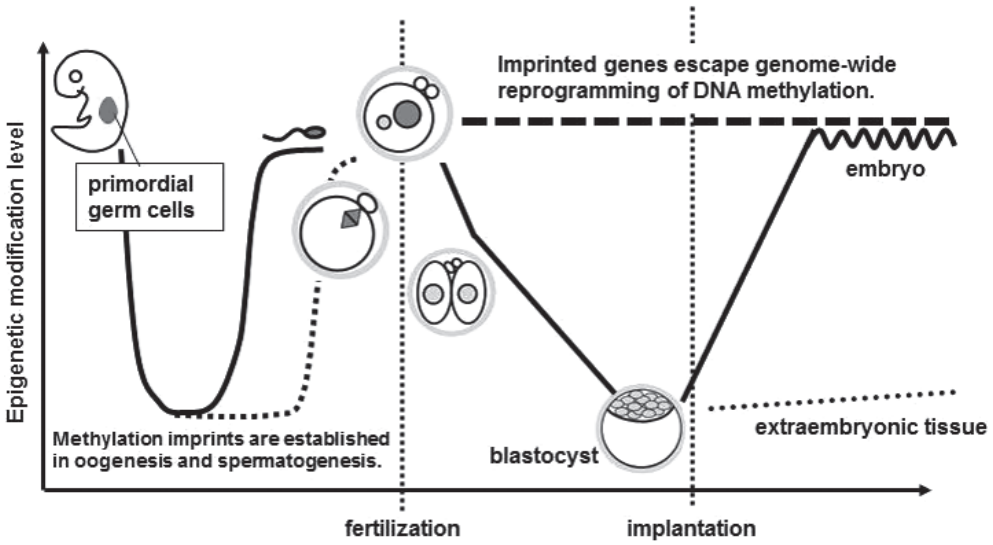

Fig. 1. Epigenetic reprograming in mammalian development.
Methylation imprints are established in oogenesis and spermatogenesis. Imprinted genes escape genome-wide reprogramming of DNA methylation.

onic tissues (placentas) maintain hypomethylated status for the whole genome. However, DNA methylation mechanism is definitely indispensable to placentation. For example, permeability and proliferation activity of trophoblasts are suppressed by 5-aza-2'-deoxycytidine which is an inhibitor of DNA methylation in cell strain and cause hypoplasia of the placentas in rats consequently [2–4]. In addition, DNA methylation states of several genes in normal placenta are similar to that of tumors, and it could create various characteristics of the trophoblasts [5].

Genomic imprinting

Genomic imprinting is an essential epigenetic mechanism especially for differentiation and growth of placentas and fetuses. Several special chromosomal regions are methylated in a completely opposite manner depending on whether their origins are paternal (a sperm cell) or maternal (an oocyte). Since the special methylation state regulate expression of neighboring genes, one allele in the region is expressed and another is silenced precisely. These special DNA methylation states are established in gametogenesis of parental generation. This is the reason why this special gene regulation is called genomic imprinting. The imprinting information is "imprinted" in every parental generation, and these genes are controlled according to "imprinted" information in next generations (Figs. 1 and 2).

If two uniparental chromosomes present, imprinted genes in the chromosomes are unbalanced in a DNA methylation state, gene expression levels become zero or double. Mouse androgenotes which posse normal number chromosomes but all are paternal origins, show massive growth placentas and poor growth embryos. In human, the paternal diploids show similar aberrant differentiation. They differentiate into trophoblasts, but never into embryonic part, and cause hydatidiform moles consequently. On the other hand, maternal diploids show abnormal differentiation in an opposite way. They differentiate into various tissues and cells but never into trophoblast lineage. Embryos derived from oocytes of Dnmt3L null mutant female mice are embryonic lethal even though they harbor a wild type *Dnmt3L* allele. Loss of specific methylation in imprinted locus in oocytes of null mutants cause loss of imprinting in zygotes and placentation failure [6, 7]. In addition to many studies of imprinting diseases and model mice, the comparison with the other species strongly suggest that the genomic imprinting is a function acquired in the evolution of the mammalian, especially placentation approximately 150 million years ago. [8, 9]

During early human development, placentas still express imprinted genes randomly and gradually they establish uniparental allelic expression (genomic imprinting) [10–12]. On the other hand, some regions strictly keep imprinting without variations of individuals and differences of developmental stages [13]. These findings are necessary for understanding molecular mechanisms of placentation, variations of normal placentation to diagnose aberrant epigenetic regulation of human development.

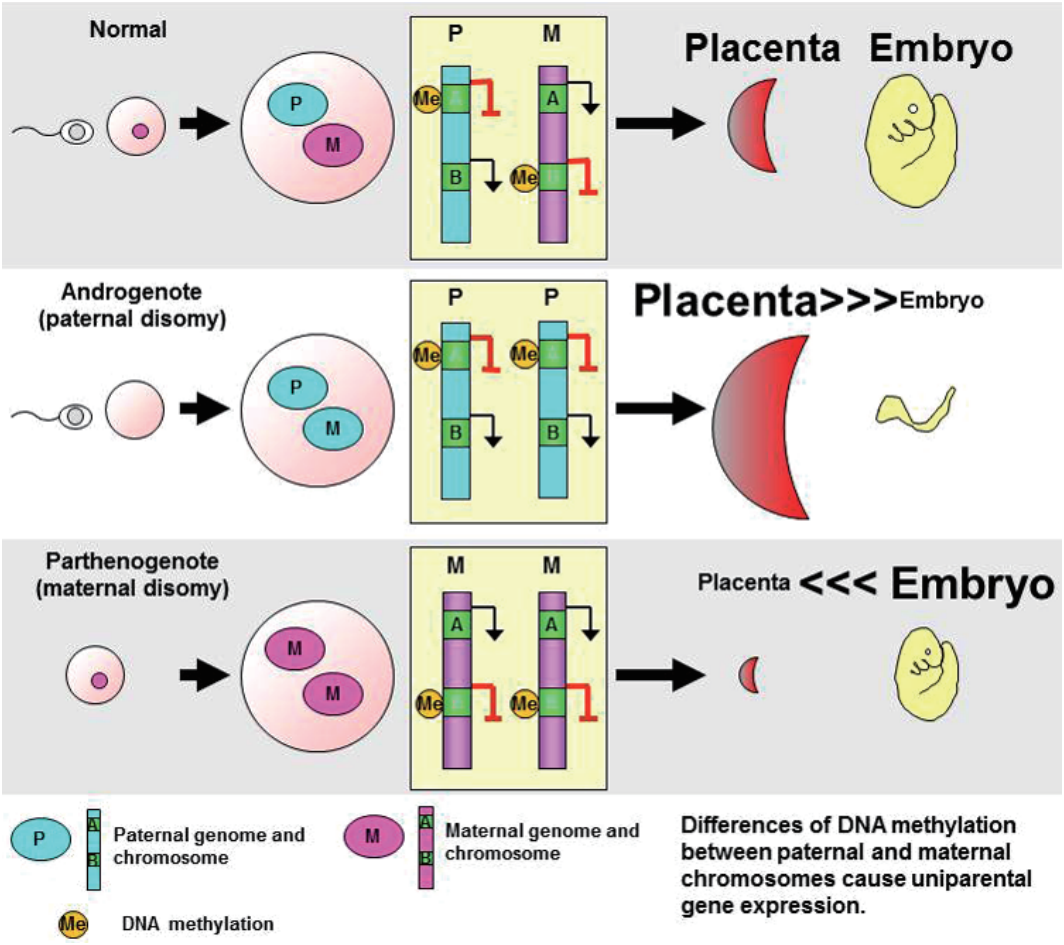

Fig. 2. Roles of genomic imprinting in development.
Differences of DNA methylation between paternal and maternal chromosomes cause uniparental gene expression.

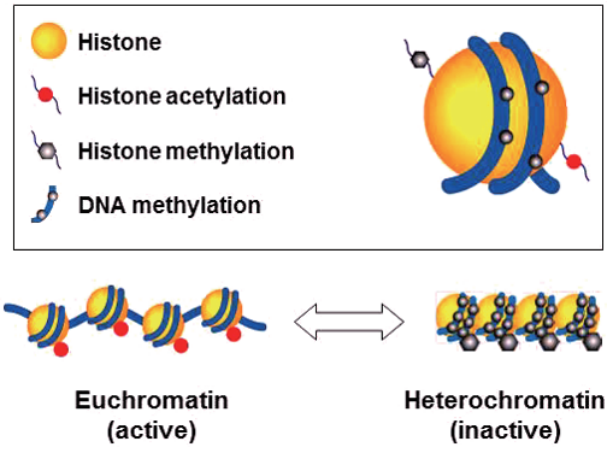

Fig. 3. Epigenetic modification and chromatin structure.
DNA methylation and histone modification (acetylation, methylation etc.) could affect chromatin structure and functions of modified regions consequently.

Histone modifications

Genomic DNA coils itself around histone proteins and it takes compact superstructure. N-terminal amino acids of histone proteins are modified by methylation, acetylation, phosphorylation and ubiquitination and affect the chromatin structure (Fig. 3). Many specific modification enzymes (i.e., SET domain proteins) and erasure enzymes (i.e., HDAC) are found. Since each histone modification has specific role on gene regulation, it is called the histone code. For example, *Eset* gene which is a methyltransferase for ninth lysine of a histone H3 tail, is necessary for retroviral suppression in genomes [14], and it is clearly shown that the Eset protein repress differentiation of ICM (Internal Cell Mass) to extraembryonic tissue in the blastocyst stage [15].

Non-coding RNA

Physiological functions of non-coding RNAs (the RNAs which is not translated to protein) becomes clear recently. non-coding RNAs are essential to the X chromosome inactivation [16] and regulate some genomic imprinting. siRNAs in oocytes are reported [17]. Such non-coding RNAs could be involved in molecular mechanisms of infertility.

Epigenetic mutations of placentas in human diseases

As it has been mentioned above, since complete hydatidiform moles are normal diploid but only possess paternal genomes, they show quite deviated differentiation potentials. Surprisingly, it has shown that familial repetitive complete hydatidiform mole cases are normal diploid with parental genomes. They are genetically normal, however, epigenetically abnormal. The mole tissues lose maternal DNA methylation imprints systematically [18]. It is expected that comparison between rare repetitive moles and typical moles/normal villus would give clear picture of the epigenetic mechanisms involved in normal development of trophoblasts.

In gestational trophoblastic diseases, various aberrant DNA methylation patterns are observed. Hypermethylation of tumor suppressor gene promoters in complete hydatidiform moles and choriocarcinoma are reported [19]. *Oct4* gene which is indispensable to maintain embryonic stem cells undifferentiated is highly methylated and suppressed in complete hydatidiform moles and choriocarcinoma cell lines [20].

Recently, several groups report that there are defects of genomic imprinting in FGR (Fetal Growth Restriction) cases [21–23]. They analyze DNA methylation and expression of imprinted genes of placentas and show loss of genomic imprinting (loss of methylation and/or loss of uniparental expression) in the FGR cases. We also analyze strict DNA methylation levels of imprinted regions systematically and find some FGR cases show placenta-restricted hypomethylation.

Pregnancy induced hypertension (PIH) is also a candidate disease which could be caused by epigenetic mutations. *p57* gene (a typical imprinted gene) knockout mice show PIH-like symptoms [24]. In PIH cases, potential causes of suppression of another imprinted gene *H19* and expression changes of micro RNAs are suggested [11, 25].

Future Directions

Epigenetics is a fundamental mechanism of gene expressions and regulate cell functions. It has been shown that several abnormal pregnancies have abnormal epigenetic background which could be causes of diseases. Epigenetic modification could be changed by environmental conditions. The changed epigenetic modification could exist and affect gene functions for long time [26]. To understand epigenetic mechanisms, genetic analysis is also important. These are two sides of the same coin. With progress of techniques for genetic analysis, it becomes easier to detect genetic variations and integrated understanding of both genetics and epigenetics profiling should advance rapidly. Epigenetics has many and varied potential medical applications. Repressors of DNA methylation and histone deacetylase is marketed as a therapeutic drug. Elucidation of epigenetics in the differentiation of trophoblast cells would propose a new concept, diagnosis and treatment for abnormal placentation in future.

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