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Epigenetics: Definition, Mechanisms and Clinical Perspective

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Abstract

A vast array of successive epigenetic modifications ensures the creation of a healthy individual. Crucial epigenetic reprogramming events occur during germ cell development and early embryogenesis in mammals. As highlighted by the large offspring syndrome with in vitro conceived ovine and bovine animals, any disturbance during germ cell development or early embryogenesis has the potential to alter epigenetic reprogramming. Therefore the complete array of human assisted reproductive technology (ART), starting from ovarian hormonal stimulation to embryo uterine transfer, could have a profound impact on the epigenetic state of human in vitro produced individuals. Although some investigators have suggested an increased incidence of epigenetic abnormalities in in vitro conceived children, other researchers have refuted these allegations. To date, multiple reasons can be hypothesized why irrefutable epigenetic alterations as a result of ART have not been demonstrated yet.

Keywords

Epigenetics; X-chromosome inactivation; imprinting; transgenerational inheritance

DEFINITION

Conrad Waddington introduced the term *epigenetics* in the early 1940s.[1] He defined epigenetics as ‘‘the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being.’’[2] In the original sense of this definition, epigenetics referred to all molecular pathways modulating the expression of a genotype into a particular phenotype. Over the following years, with the rapid growth of genetics, the meaning of the word has gradually narrowed. Epigenetics has been defined and today is generally accepted as ‘‘the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence.’’[3] The epigenetic modifications described in current literature generally comprise histone variants, posttranslational modifications of amino acids on the amino-terminal tail of histones, and covalent modifications of DNA bases. The validity of the current definition of epigenetics should be seriously

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questioned because the previously mentioned epigenetic modifications also have a crucial role in the silencing and expression of noncoding sequences.

THE NATURE AND INHERITANCE OF EPIGENETIC MARKS

In addition to their importance in the commitment of cells to a particular mitotically inheritable form or function, epigenetic marks have a crucial role in guaranteeing genomic stability. Indeed, the silencing of centromeres, telomeres, and transposable elements (TEs) ensures the correct attachment of microtubules to centromeres, reduces excessive recombination between repetitive elements, and prevents transposition of TEs and resulting insertional mutagenesis.[4–6]

Although covalent modifications of DNA bases have been described since 1948,[7] it was only in 1969 that Griffith and Mahler suggested that these modifications may modulate gene expression.[8] The predominant modification in mammalian DNA is methylation of cytosine,[7] followed by adenine and guanine methylation.[7,9] Although methylation of cytosine bases in mammalian DNA has been primarily described in the context of CpG dinucleotides,[10] evidence suggests that cytosines in non-CpG sequences are also frequently methylated.[11–13] Because the promoter regions of silenced genes possess significantly more methylated cytosines in comparison with actively transcribed genes, this modification has been implicated in transcriptional repression.[14,15] Methylation of cytosine in the promoter region may repress gene expression by preventing the binding of specific transcription factors[16] or may attract mediators of chromatin remodeling, such as histone-modifying enzymes or other repressors of gene expression.[17–20] In mammals, the mitotic inheritance of methylated DNA bases is primarily ensured by a maintenance of DNA methyltransferase (DNMT1),[21–23] whereas DNA methylation enzymes DNMT3A and DNMT3B are mainly responsible for de novo methylation of unmethylated sites.[24] Various studies have shown that DNMT3A and DNMT3B target different sites for methylation depending on the cell type and the stage of development.[6,25,26] De novo methyltransferases may be directly targeted to specific DNA sequences, may necessitate the interaction with other DNA binding proteins or may be guided by RNA interference (RNAi) in a process called RNA-directed DNA methylation (RdDM).[27]

Besides covalent modifications of DNA, histones and their posttranslational modifications have also been implicated in the organization of chromatin structure and regulation of gene transcription. Generally, histone classifications comprise the main histones or their variants H1, H2A, H2B, H3, and H4.[28–31] The fundamental building block of chromatin is the nucleosome and consists of DNA spooled around an octamer of histones. Each octamer contains two units of each principal or variant histone H2A, H2B, H3, and H4.[32] Linker DNA connecting nucleosomes associates with the main form or variants of the linker histone H1. A variety of histone-modifying enzymes is responsible for a multiplicity of posttranslational modifications on specific serine, lysine, and arginine residues on the amino-terminal tail of these histones.[33,34]

The correlation of specific posttranslational modifications on the histones with transcriptional events has resulted in the histone code hypothesis.[35] To date, the best characterized modifications are acetylations and methylations of lysine residues on histones H3 and H4. Although all acetylations of lysine residues on H3 and H4 have been associated with transcriptional activation (H3K9, H3K14, H3K18, H3K23, H4K5, H4K8, H4K12, and H4K16),[36–41] methylation of lysine residues may be either associated with transcriptional repression (H3K9, H3K27, and H4K20) or activation (H3K4, H3K36, and H3K79) depending on which amino acid and to what extent (monomethylation, dimethylation, or trimethylation) the residue is modi-fied.[41] Although not as well documented, it has become clear that posttranslational modifications of other histones also have an important role in chromatin

structure and gene regulation. Indeed, more recently it has been reported that mutations on specific sites of histones H2A and H2B modify the transcription of various genes.[42,43] Similarly, as for DNA methylation enzymes, histone-modifying enzymes may be targeted to specific DNA sequences directly[19,20] or may necessitate the interaction of intermediates such as Polycomb and Trithorax group proteins and/or RNAi.[44–47] In contrast to DNA methylation, it is unclear how and if histone modifications are correctly replicated during mitosis. Although a few investigators have claimed that histone complexes are distributed semiconservatively over the replicated genome,[48] most researchers have refuted this manner of histone deposition.[49] As a result, it should be questioned whether covalent histone modifications and histone variants are epigenetic marks according to the current definition of epigenetics.

X-CHROMOSOME INACTIVATION AND AUTOSOMAL IMPRINTING

During evolution, an alteration or acquisition of a sex-determining gene on one copy of a pair of chromosomes has resulted in the emergence of sex chromosomes. Consequently, the sexes are generally determined by the presence of a hetero- or homomorphic pair of allosomes. With time, as a result of reduced recombination events between these heteromorphic chromosomes, vastly dissimilar sex chromosomes have arisen. This dissimilarity between the allosomes is at the origin of a gene dosage inequality between the two different sexes.[50] To remediate to this imbalance, many species have adopted gene dosage compensation mechanisms. The epigenetic gene dosage compensation mechanisms of genes located on the sex chromosomes vary with species, from simple transcriptional modulation to the entire silencing of one allosome.[51] Although it is generally accepted that therian mammals (placentals and marsupials) equalize X-chromosome gene dosage between the sexes by inactivating one X chromosome in females, it has also been suggested that transcription from the active X chromosome is upregulated to maintain balance between autosomal and allosomal gene expression.[52] Initially, the observation that female mice heterozygous for X-chromosome-linked coat color genes displayed a mosaic phenotype led to Mary Lyon's hypothesis that either the paternally or maternally derived X chromosome could be inactivated in female animals.[53] Later investigations revealed that this pattern of X-chromosome inactivation may differ depending on the species and the developmental status of the conceptus. Indeed, female offspring from placentals always possess a mixture of cells with an inactive X chromosome from either maternal or paternal origin, whereas marsupial offspring only present inactive X chromosomes from paternal origin.[54,55] In addition, though random X-chromosome inactivation is reported in embryonic lineages from mouse postimplantation embryos, the paternally inherited X-chromosome is always preferentially silenced in preimplantation embryos[56] and the resulting extraembryonic lineages.[57] This latter form of X-chromosome inactivation is commonly referred to as imprinted X-chromosome inactivation. Although the ultimate outcome of both random and imprinted X-chromosome inactivation is the silencing of one X chromosome, studies suggest that the maintenance of epigenetic marks on the inactive X chromosome is markedly determined by whether the X chromosome underwent random or imprinted inactivation. Indeed, the silencing of imprinted inactive X chromosomes mainly depends on histone modifications applied by Polycomb proteins rather than DNA methylation, whereas DNA methylation is a crucial factor for the maintenance of the inactive state of randomly inactivated X chromosomes.[58,59] To date no conclusive evidence exists for imprinted X-chromosome inactivation in human conceptuses.[50]

To permit random X-chromosome inactivation in the embryonic lineage of mice, a reactivation of the initially silenced X chromosome is necessary. Random X-chromosome inactivation is controlled by a region on the X chromosome called the X inactivation center (XIC). The XIC possesses the genes *Xist* and *Tsix*, which contain noncoding RNAs that are crucial for inactivating and maintaining activity of specific X chromosomes. Indeed, transcription of *Xist* on the inactive X chromosome mediates its silencing, whereas *Tsix* transcription from the

active X chromosome prevents its inactivation.[60] Although it remains unknown how X chromosomes are randomly selected for activity or inactivity, three mechanisms have been proposed for the selective silencing of the paternally derived X chromosome during early fetal development. Conceptually, the paternal X chromosome can enter the oocyte in a preinactivated condition or may be selectively silenced after fertilization.[51] Meiotic sex chromosome inactivation (MSCI) during spermatogenesis supports the view that the paternal X chromosome can be inherited in an inactive state.[61] However, it has also been claimed that MSCI is not crucial for imprinted X-chromosome inactivation because autosomes that do not undergo MSCI, but present *Xist* transgenes, are also preferentially silenced when paternally inherited.[62] In opposition to the inheritance of a preinactivated X chromosome, the differential remodeling of the paternal and maternal chromatin and/or the translation of specific parental imprints on the X chromosomes after fertilization may be at the origin of the initial selective inactivation of the paternal X chromosome in female embryos. Indeed, *Xist* transcription may be instigated on the paternally derived X-chromosome as a result of the exchange of protamines in the paternal pronucleus with histone variants favoring transcription.[63] Alternatively, imprinted X-chromosome inactivation has also been shown to be dependent on various differential epigenetic imprints on *Xist* and *Tsix* genes acquired during male and female germ cell development.[64,65] In brief, X-chromosome inactivation in mammals has originated to compensate a gene dosage inequality between the two different sexes. Because of its necessity, the establishment and maintenance of X-chromosome inactivation seems to be controlled by a variety of redundant epigenetic marks and mechanisms.

Pronuclear transfer experiments in the early 1980s revealed that mammalian reproduction necessitates the contribution of a paternal and maternal genome to be successful.[66,67] The preferential mono-allelic expression of specific genes from either the maternal or paternal allele was believed to be at the origin of this phenomenon. The first imprinted genes in mammals were identified in the early 1990s.[68–70] Genomic imprinting has been observed in angiosperms and mammals and would have independently evolved in these two taxa as a result of selective pressure on specific genes.[71] Although many genes remain imprinted throughout the entire life of an organism, some genes are imprinted in a tissue-specific or temporal manner, similarly to the *Xist* gene. Imprinted genes are organized in clusters or domains, and their expression is under control of a cis-acting imprinting control element (ICE).[72] Similarly to the XIC region on the X chromosome, ICE elements on autosomes acquire differential imprints during germ cell development, depending on their parental origin. Like X-chromosome imprints, autosomal imprints in female mammals are established during folliculogenesis, whereas imprints in males are reset during fetal development.[73–78] The fact that the imprinted inactivation of the paternal X chromosome and autosomal genes present many molecular similarities has led to the hypothesis that these phenomena have coevolved.[79]

TRANSGENERATIONAL INHERITANCE

Although the maintenance, as well as the erasure, of acquired epigenetic marks between generations has both beneficial and deleterious effects, it is unknown to what extent epigenetic marks are maintained or erased between generations in mammals. Because primordial germ cells are set aside during mammalian fetal development and because of epigenetic reprogramming events during germ cell development and early embryogenesis, acquired epigenetic states are believed to be rarely passed on to progeny.[80] The erasure of epigenetic marks occurs in female and male mammals during primordial germ cell development and early embryogenesis, whereas the acquisition of epigenetic marks takes place at different times during female and male gametogenesis. Indeed, epigenetic marks in female germ cells are established during folliculogenesis, whereas male germ cells acquire their epigenetic marks during fetal development.[73–78] The fact that imprints are maintained during early embryogenesis highlights that some sequences may escape reprogramming events. Stella is

among a group of proteins that may play an important role in the suppression of epigenetic reprogramming of these specific sequences.[81] The failure to erase epigenetic marks during primordial germ cell development or subsequent early embryogenesis is at the origin of transgenerational inheritance of epigenetic traits. A clear example of a gene susceptible to transgenerational inheritance is the *Agouti viable yellow* (A^{vy}) allele in mice.[82] The variable epigenetic status of an intracisternal A particle element (IAP) located upstream from the coding region of A^{vy} in mice is responsible for the variable expression of this allele in adult mice. As a result of incomplete erasure of epigenetic marks on IAPs, this variable expression is often transgenerationally inherited by offspring.[82] Evidence suggests that many IAPs fail to undergo epigenetic reprogramming during germ cell development.[83] The high incidence of IAPs in mammalian genomes has consequently led to the belief that this type of transgenerational inheritance may be more prevalent than initially conceived.

CLINICAL IMPLICATIONS OF EPIGENETIC ALTERATIONS

Given the extent of epigenetic reprogramming that occurs during gametogenesis and embryogenesis and the vulnerability of the process, it is not difficult to understand how alteration in reprogramming could be of clinical relevance. Because epigenetic reprogramming occurs during folliculogenesis and embryogenesis, any disturbance of the normal natural environment during these critical phases could cause epigenetic alterations. Accordingly, researchers have attempted to determine whether children conceived using assistive reproductive technology (ART) carry epigenetic reprogramming defects. A review of an association of ART and epigenetic alterations is covered in detail in articles later in this issue. Importantly, although the whole genome is reprogrammed during germ cell development and embryogenesis, it should be noted that to date only a limited number of loci have been investigated. These loci generally comprise genes in which their epigenetic status significantly affects a perceptible phenotype. Although a specific clinical phenotype has not yet been associated with an epigenetic change, it is it possible that pathology may emerge from a not yet recognized epigenetic alteration.[84] An excess of epigenetic alterations could have an immediate impact that precipitates pre- or postnatal death.

At the other extreme, an epigenetic change might result in a perceptible alteration later in life such as cancer, coronary heart disease, stroke, or diabetes. An increased risk of heart disease, stroke, and diabetes is associated with malnutrition in utero and low birth-weight.[85] Again, the role of nutrition and diet during pregnancy is covered in detail in ensuing articles in this issue, but it must be considered whether children of ART with a low birthweight could have a predisposition for these chronic phenotypes. Concerns have also been raised about the epigenetic status of tumor suppressors or fertility concerns in individuals exposed to environmental toxins. Subsequent articles address this issue in greater depth as well, but there is sufficient evidence in animals to warrant concern.

In conclusion, there is reason to suspect that early development is vulnerable to unwanted changes in epigenetic inheritance. Animal studies have shown that epigenetic reprogramming is a fragile process that is easily modified,[86–91] and such data provide compelling biologic plausibility for clinical concern. Although animal models may provide some information, the results may not always be representative of the epigenetic events that occur in humans. Because of the potential for adverse health effects in offspring conceived using ART and in children born from altered nutritional states in pregnancy or exposed to environmental toxins, further research is needed.

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