

Epigenetic vulnerability and the environmental influence on health

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Abstract

A growing body of evidence has drawn the attention of the scientific community by indicating the potential vulnerability to environmental changes of epigenetic mechanisms that control gene expression. Being critical components of normal development, the importance of epigenetic mechanisms for normal biology is illustrated by the fact that abnormal epigenetic patterns have increasingly been linked to the aetiology of various diseases including cancer, paediatric syndromes, autoimmune diseases, genetic disorders and even the molecular process of ageing. It is estimated that the degree of vulnerability to changes in epigenetic patterns is high during early embryonic development, a period of life in which epigenetic patterns are established and cell differentiation is intense. Moreover, increasing amounts of relevant data and information reveal that the environment might potentially impact on epigenetic patterns at every period of life. Within this context, in this study we will review the principles of epigenetic vulnerability to environmental changes, the impacts on development, the association with the origin of common diseases and also speculate about the potential of lifestyle changes to modulate epigenetic patterns and contribute to preventing common diseases.

Keywords: Epigenetics, DNA methylation, histone modification, environmental influence on health

Experimental Biology and Medicine 2013; **238**: 859–865. DOI: 10.1177/1535370213490630

Background

The past decades have undoubtedly been marked by the advances in the understanding and knowledge of genome sequences and gene functions; however, still today it is not an easy task to answer some questions concerning the influence of the environment on health, for example, how can lifestyle impact on gene expression and lead to diseases? Or, how can lifestyle impact on gene expression and contribute to preventing the onset of diseases? Within this context, epigenetic mechanisms are emerging from the literature accompanied by intriguing propositions for interaction between gene expression, environment and phenotypic changes, independently of changes in the DNA sequence.

The term ‘epigenetics’ has been commonly used by geneticists to describe the mitotically inheritable changes that control gene expression without changing the original DNA sequence.^{1–3}

In molecular terms, epigenetic mechanisms are generally divided into two types: (1) covalent modifications at the N-terminus tails of histone proteins and (2) methylation of carbon 5 of cytosine, generally followed by guanine (CpG nucleotides), in DNA molecules.^{4,5}

Histone consists of small proteins that form spools for wrapping DNA into nucleosomes. Nucleosomes are formed by eight histone molecules, two of each histone H2A, H2B, H3 and H4, around which a loop of approximately 146pb DNA is wrapped. Post-translational modifications of histone tails (i.e. strings of amino acids that protrude outside the nucleosome) are dynamic and reversible processes that attach or remove chemical groups in a site-specific manner. In theory, any histone modification might disrupt the electric charge of the amino acid residues and affect the interactions between histones and the DNA backbone. As far as we know, the most important histone modifications associated with gene expression controls include acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation and others.⁶

A cascade of molecular mechanisms and interactions is generally triggered by histone modifications. The events that link acetylation of lysine residues on histone tails to changes in gene expression are among the best elucidated. Stated in more simple terms, the introduction of an acetyl group by histone acetyltransferases neutralizes the positive electric charge of lysine leading to loss of its electrostatic attraction to the negatively charged phosphate of the DNA backbone.

This modification consequently weakens the binding of DNA to histones, thereby assembling an unfolded chromatin and facilitating the access of transcriptional machinery and gene expression.⁶

DNA methylation is certainly the most intensely studied epigenetic modification. Dynamic, with a potential to change during development, DNA methylation exerts a fundamental role in the establishment and control of important biological processes, such as cell differentiation in embryo development, X chromosome inactivation in females, genomic imprinting, suppression of endogenous retroviruses and chromosomal stability.^{7–12} In the course of the DNA methylation process, the methyl radical (CH₃) is obtained from the donor S-adenosyl-methionine (SAM) and transferred to cytosine by a family of DNA methyltransferases (Dnmts). A group of Dnmts with different activities have been identified to date: (1) Dnmt1 is responsible for maintaining the methylation status during cell replication by adding a methyl group to a hemimethylated DNA chain¹³; (2) Dnmt2, despite initial evidence indicating enzyme inactivation, was found to develop tRNA methyltransferase activity, thereby creating the possibilities of this protein to play biological roles that might be broader than previously thought¹⁴ and (3) Dnmt3s (Dnmt3a, Dnmt3b) which methylates previously unmethylated DNA (*de novo* methylation).¹⁵ Other enzymes that also have no methyltransferase activity of their own, but share homologies with Dnmt3s, such as Dnmt3L (Dnmt3-Like), play an essential role in the establishment of tissue-specific methylation imprints and are included in the group of Dnmt3 enzymes.^{16,17}

Preliminary evidences indicated that DNA methylation exerts its effects through altering the interactions of transcription factors and/or proteins that specifically bind to methylated DNA (due to a methyl-binding domain – MBD); however, recent data have shown that the functions of DNA methylation might be dependent on the genomic context and much more complex than previously thought. For example, whereas methylation at CpG might inhibit the binding of transcription factors to its recognition sites leading to gene silencing, for other genomic loci the methylated dinucleotides can be targeted by MBD-proteins that recruit additional complexes which include histone deacetylases leading to a closed chromatin configuration, thus ensuring allele-specific gene inactivation.¹⁸ Alternatively, DNA methylation can: interfere with interactions between various trans-acting factors, impact on splicing mechanisms, alter the activity of enhancers and insulators, and also exert an important role in the control of genome stability.¹⁹ Despite the enormous advances obtained in this field during the past decades various questions still remain unclear and future studies are needed for a complete understanding of the role of DNA methylation.

Early life and the environmental impact on epigenetic programming

A complex phenomenon of epigenetic programming characterized by modifications of DNA methylation pattern and

histone modifications that are crucial for the control of developing events, such as the establishment of totipotency of stem cells, cellular fate and early organogenesis takes place at the beginning of embryo development in mammals.^{20,21}

A few hours after fertilization, the male and female pronuclei undergo a reprogramming process marked by a wave of genomic demethylation which is important for establishing the totipotency and the reprogramming of somatic cells of the embryo gene expression. However, some specific genomic loci are protected against this demethylation wave and maintain the parental (gamete) pattern of methylation in somatic cells. The protected loci correspond to differentially methylated regions (DMRs) (methylated only in the paternal or maternal inherited allele) involved in the control of genomic imprinting.²²

Genomic imprinting is an epigenetically controlled mechanism in which gene expression depends on the parental origin and only one of the two alleles (paternally or maternally inherited) is expressed. At a functional level, imprinted regions are effectively haploid, which makes them vulnerable to recessive mutations and epigenetic changes. Approximately, 85 imprinted genes have been identified to date, with most being associated with the control of fetal growth, the transfer of nutrients from mother to fetus through the placenta and brain functions. Generally, imprinted genes are mapped to clusters and at least one DMR is included among them, suggesting the existence of a similar control mechanism for the various imprinted regions.^{22,23}

Acquisitions of the methylation patterns of male and female differentially methylated regions (DMRs) occur during gametogenesis at developmentally different stages. For example, DNA methylations at the paternal loci *H19*DMR, *RAS* protein-specific guanine nucleotide-releasing factor 1 (*Rasgrf1*) and *gene trap locus 2* (*Gtl2*) in male germ lines are initiated during germ cell development in embryogenesis and are completed by the pachytene phase of post-natal spermatogenesis in mice.^{24–26} On the other hand, methylations at the maternal loci, *insulin-like growth factor 2 receptor* (*IGF2R*), *small nuclear ribonucleoprotein N* (*Snrnp1*), *paternally expressed 1* (*Peg1*) and *paternally expressed 3* (*Peg3*) in female germ lines are acquired asynchronously in a gene-specific manner only during the oocyte maturation in the postnatal growth phase.²²

The Beckwith-Wiedemann syndrome (BWS) is prototypic of genomic imprinting disease characterized by overgrowth, macroglossy, abdominal wall defects and predisposition to embryonal cancer (mainly Wilms tumour in the kidney).²⁷ The aetiology of BWS is attributed to epigenetic alterations in a cluster of imprinted genes mapped to human chromosome 11p15.5. This imprinted cluster is divided between centromeric and telomeric regions. The telomeric region harbours the paternally expressed *IGF2* gene and the maternally expressed *H19* gene, whereas the centromeric region harbours the paternally potassium voltage-gated channel 1 opposite strand/antisense transcript 1 (*KCNQ1OT1*) and the maternally expressed *cyclin-dependent kinase inhibitor 1C* (*CDKN1C*) and *potassium voltage-gated channel 1* (*KCNQ1*) genes.

Imprinted gene expression patterns at the telomeric and centromeric regions are controlled by the differential methylation of the H19DMR and KvDMR, respectively. H19DMR is commonly methylated on the paternally inherited allele, whereas KvDMR is methylated on the maternal allele.^{28,29} Approximately, 20% of all BWS cases result from paternal uniparental disomy, whereas paternally derived duplications and maternally derived translocations of 11p15.5 account for approximately 2% of the cases. Mutations in the maternally derived allele of the *CDKN1C* gene (also known as *p57^{kip2}*) are found in approximately 10% of sporadic cases. Aberrant methylation at the paternal imprinting control region (ICR) H19DMR have been found in approximately 2–7% of cases and the loss of methylation at the maternal allele of KvDMR ICR – an intronic CpG island within *KCNQ1* gene – is the most frequent alteration found in approximately 50% of cases. Aetiology is unknown for 10–15% of BWS patients.³⁰

Early studies in mouse models have drawn the attention of the scientific community by providing evidence of vulnerabilities of embryo reprogramming of genomic imprinting to changes in the embryo culture medium.^{31–33} The abovementioned association between *in vitro* reproductive technologies and epigenetic alterations was confirmed by epidemiological studies containing alarming data, reporting a 4–9 fold increased risk for BWS observed in children conceived by assisted reproductive technologies (ART) when compared to naturally conceived.^{34–38} In theory, the association between epigenetic alterations and *in vitro* reproduction is based on the fact that some procedures used in ART, such as gamete handling and embryo culture, take place at two biological moments that are of vital importance in epigenetic programming: gametogenesis and postfertilization.

Superovulation, a common procedure of IVF protocols, has been demonstrated to induce the formation of oocytes without their correct primary imprint in both human and mouse models, thereby providing support to the proposition that hormonal induction of the ovulation with high doses of gonadotrophins might generate epigenetically ‘immature’ oocytes with incomplete methylation acquisition.³⁹

On the other hand, observations that abnormal methylations in BWS patients conceived by ART are not confined to the 11p15 or to a specific parental inheritance serve as an argument in favour of the hypothesis that an epigenetic error might occur after fertilization, most likely due to a failure in maintaining parental-specific methylation marks.⁴⁰

In a report of a child with BWS and that had been conceived by ICSI, our group contributed with data reinforcing the possibility and importance of the involvement of post-fertilization vulnerability of epigenetic mechanisms by demonstrating an abnormal methylation pattern at the KvDMR exclusively in cells from embryonic origin and not in cells from extra-embryonic origin.⁴¹

In addition, contrary to previous concepts of association between epigenetic alterations and phenotypic changes, we have brought forward evidence of abnormal methylation patterns at KvDMR in clinically normal children conceived

by ART.⁴² Also, we provided further evidence of discordant methylation patterns between dizygotic twins conceived by ART, a fact that leads us to hypothesize that: (1) different embryos might present differential vulnerability to environmental exposure (embryo discordant vulnerability) or (2) alteration in the methylation pattern might have occurred during gametogenesis resulting in the production of some oocytes without the correct primary imprint at KvDMR1 (oocytogenesis vulnerability).

The association between ART and abnormal epigenetics is also evidenced in bovine models conceived by IVF, with increased incidence of the large offspring syndrome (LOS), a pathology characterized by high birth weight, respiratory problems and an increase in prenatal death, caused by loss of imprinting of the *IGF2R* gene, is commonly observed.⁴³

Comparative analysis of epigenetic errors occurring in consequence of *in vitro* fertilization in both human and bovine models is important to exclude the influence of the parental genetic background on the origin of the epigenetic alterations, since, contrary to humans, in whom IVF is an alternative to fertility problems, in bovines the best parental genetic backgrounds are chosen and IVF is generally used as a tool for genetic improvement of offspring and genetic gain. Thus, the observation of LOS in bovine models provides clear evidence that epigenetic abnormalities consequent to *in vitro* reproduction are not dependent on an impaired parental genetic background or the cause of infertility, highlighting the influence of the procedures used in assisted fertilization (such as the gamete handling and embryo culture) on epigenetic acquisition or maintenance.

In addition to epigenetic changes, ART has also been associated with abnormal behaviour and brain functioning in animals. Long-term alterations in behaviour, such as anxiety, locomotor activity and spatial memory were previously linked to the culture of pre-implanted embryos.⁴⁴ Intriguingly, the behavioural variances, observed in mice are not consequent to individual ‘aberrant’ animals, suggesting a possible association between ART and abnormal epigenetic patterns in specific areas of brain. Extrapolation of this animal evidence to the human domain is still underestimated and future studies may identify and explain the possible involvement of ART in human behavioural abnormalities through epigenetic changes.

During the DNA methylation process, the methyl group is obtained from the methyl donor SAM by Dnmt enzymes, which convert SAM to S-adenosylhomocysteine. SAM levels are dependent of dietary intake, since mammals cannot synthesize major sources of one-carbon unit methionine and choline, or critical co-factors for methyl group metabolism, such as folic acid, vitamin B₁₂ and pyridoxal phosphate. Thus, an unbalanced ratio (excess or deficit) of the major sources of one-carbon units and co-factors for methyl group metabolism may alter the supply of methyl groups and consequently establishes links between nutritional aspects and epigenetic alterations.⁴⁵

The viable yellow agouti (*A^{vy}*) is an interesting mouse model that has been comprehensively explored over the past few years to demonstrate the effects of gestational

maternal nutritional factors and characteristics in the epigenetic programming of offspring. Basically, the agouti gene is genetically and epigenetically regulated by an intracisternal A particle retrotransposon insertion which contains a promoter that drives ectopic agouti expression and affects coat colour. Using the A^{vy} model, Waterland and Jirtle⁴⁶ demonstrated that the excess of methyl donor supplementation before and during pregnancy can alter the methylation pattern at specific loci indicating a direct influence of maternal nutrition on the offspring's epigenetic mechanisms.⁴⁶

In humans, the association between early life and the origin of late-onset diseases lies at the basis of the burgeoning 'Developmental Origin of Health and Disease (DOHaD) Theory'. Proposed in the late 1980s, the DOHaD theory basically states that during normal early development, organs and body systems go through 'critical' periods, during which they are plastic and sensitive to the environment and, depending on the degree of adaptation of the body during these periods, this phenomenon can manifest itself as diseases in adult life.⁴⁷

The scenario of nutritional deficiencies and starvation that affected part of the Dutch population at the end of the World War II – a 24-week period during which people officially consumed around 900 kcal/day – provides one of the best existing human models to assess whether exposure to famine during pregnancy is related to adult diseases. According to epidemiological studies involving this group of individuals, gestational nutrition deficiency is associated with increased prevalence of overweight, hypertension, coronary heart disease, major affective disorders, antisocial personality disorders, schizophrenia, decreased intracranial volume and congenital abnormalities of the central nervous system.^{48–50}

Epigenetic mechanisms are the principal candidates for the biological bridge that links early life to late phenotypes in DOHaD. Corroborating this association, individuals who had been prenatally exposed to the Dutch Famine had, six decades later, less DNA methylation of the imprinted *IGF2* gene when compared to their unexposed same-sex siblings, confirming the vulnerability of epigenetic mechanisms during early development and also indicating that epigenetic alterations that occur during early development persist over the years.⁵¹

The vulnerability window of embryo epigenetic programming and the late-onset effects have been supported by various pieces of evidence in animal models. For instance, *in utero* exposure to ethanol and bisphenol A (BPA) – a chemical product used in the manufacture of polycarbonate plastic and methylmercury have previously been demonstrated to affect the epigenome of the developing embryo, culminating into increased predisposition to adult disease.^{52–54} Little is known about the molecular mechanism by which BPA and methylmercury affect epigenome. More specifically in the case of ethanol, exposure to this compound has been demonstrated to affect DNA methylation by interfering with the expression of Dnmt3 genes or by an antagonistic effect on folate methyl group metabolism.^{55–57}

Lifestyle and health: an epigenetic perspective

Good health practices certainly have an impact on cell homeostasis and are reflected in the prevalence of common diseases in humans. For that reason, investigating the exact molecular pathways that link lifestyle to the aetiology of diseases continues to be one of the main focuses of modern medicine.

Epigenetic mechanisms have emerged with increasing prominence as disease-related factors in the medical literature over the past decades. Cancer was the first human disease linked to epigenetic alterations, and when in 1983 widespread loss of DNA methylation was observed at first time in a colorectal cancer, this was followed by an extraordinary increase in the number of studies investigating this association.⁵⁸

In general, cancer epigenetics can be divided into two types: *loci*-specific hypermethylation and global DNA hypomethylation. Loci-specific hypermethylation is directly linked to gene inactivation (preferable of tumour suppressor genes), whereas the loss of global DNA methylation is generally associated with increased chromosomal instability and abnormal expression of oncogenes.⁵⁹

Recent evidences also suggest that there may exist an association between epigenetic changes and the ageing process. Comparisons between fetal and adult tissues have revealed remarkably different, tissue-specific patterns of DNA methylation.⁶⁰ Overall global hypomethylation and loci-specific hypermethylation have been implicated as epigenetic hallmarks of ageing, suggesting a common molecular pathway for cancer.⁶¹

A comparative study between monozygotic twins of different ages (3 and 50 years) showed that epigenetic differences among twins develop throughout life as a consequence of differential environment exposure.⁶² Nevertheless, the questions concerning how, when and which environmental factors can induce epigenetic modifications and modulate ageing and/or predisposition to disease remain for the most part unanswered.

The links between cigarette smoking, alcohol consumption and dietary intake and genome-wide or loci-specific epigenetic changes have been intensely investigated over the past years.⁶³ However, the list of environmental factors capable of inducing epigenetic errors seems to be completely underestimated and exposure to apparently inoffensive products such as hair dye should also be taken into consideration.⁶⁴

In contradiction of expectations that the epigenetic inter-individuality is a consequence of differential environment exposures, Zhang *et al.*⁶⁵ reported in a recent study that epigenetic differences between individuals might depend more on race/ethnicity than environment. According to this study, non-Hispanic blacks have significantly lower levels of global methylation when compared to non-Hispanic whites, irrespective of age, cigarette smoking and/or alcohol drinking habits or diets.⁶⁵

It is certain that we currently have a very poor understanding of the environmental modulation of epigenetic mechanisms. The potential of simple changes in

lifestyle – such as initiating a calorie restriction diet or an aerobic exercise programme – to affect epigenetic patterns and act as preventive and restorative intervention is still totally speculative.

Practicing physical exercises certainly has numerous positive effects on brain functioning, including improved cognitive performance, neurogenesis, synaptogenesis and angiogenesis.^{66–68} The hippocampus, a part of the brain area that plays a key role in learning, memory and stress response, seems to be particularly sensitive to physical exercise.^{69–71} Recent evidence revealed that improvements in the cognitive capabilities of exercised rats, compared to sedentary counterparts, and the increased capability to face and handle psychologically stressful challenges are consequent to epigenetic changes in the dentate gyrus neurons of the hippocampus.⁷²

According to these sources, a significant higher level of global genomic DNA methylation has recently been observed in human individuals that engage in physical activities for 26–30 min/day when compared to people who engage in physical activity for only ≤ 10 min/day.⁷³ Despite the limited sample size of the data used in the aforementioned study, this information warrants further investigation of the impact of physical activities on DNA methylation. Furthermore, a recent follow-up study reported on a very interesting interrelationship between physical exercise, epigenetic changes and cancer prognostics. After studying the effects of six months of moderate-intensity aerobic exercise on DNA methylation of peripheral blood leukocytes of breast cancer survivors, Zeng *et al.*⁷⁴ showed that physical activity affects loci-specific epigenetic mechanisms that control the expression of tumour suppressor genes, such as the lethal(3)malignant brain tumour-like protein 1 (L3MBTL1), and is linked to favourable survivor outcomes.

Although the data and information on the effects of physical exercise on epigenetic mechanisms in humans are still scarce in the literature, the small number of currently available reports is sufficient to open an intriguing avenue for future studies in this field.

Conclusion

The knowledge and understanding of molecular linkages between environment and health has greatly improved by newly emerging data on epigenetic vulnerability. Future human studies addressing the potential of preventive interventions based on the modulation of epigenetic patterns should be encouraged and might reveal, at the molecular level, the role of lifestyle in lifelong good health.

Authors' contribution: MV drafted the manuscript. GG helped to draft the manuscript and revised it critically. MV and GG read and approved the final version of the manuscript.

FUNDING

This research received a grant from the National Foundation for Development of Private Education (Funadesp) (no.5300665).

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(Received June 18, 2012, Accepted February 7, 2013)