Epigenetics in relation to environmental pollution, nutrition and pathogenesis

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Abstract

The review is based on classical and modern data on genetics and epigenetics of gametogenesis and embryonic development in vertebrate including some personal results, but its main purpose was to offer the reader the most recent data collected from some representative scientific papers appeared in scientific journals or as Med-line communications concerning the interference between epigenetic mechanisms, environmental pollution, nutrition, life-style and pathogenesis in humans as it was deduced from epidemiological and clinical studies as well as from laboratory experiences using murine model.

The main idea of this review is that all modern results obtained in the field of epigenetics support the classical long-lasting controversial Lamarck’s principle of “the inheritance of acquired characters” and represent a basis for understanding the biological memory within and across generations.

Keywords: epigenetic memory, gene imprinting, environmental genome instruction, environmental exposure, nutriepigenomics, obesity, diabesity.

Introduction

Epigenetics is concerning with phenotype alterations based on specific changes in cellular properties that are transmitted from parents to the offspring without affecting the genetic information in itself (i.e. without any change in nucleotide sequence of DNA).

Epigenetics regulation of gene expression is a mechanism that is used by the organism to integrate in its genome intrinsic and environmental signals (Jaenisch and Bird, 2003).

Involvement of epigenetic mechanisms in biological processes might be the only way to accommodate that controversial lamarckian “principle” of “the inheritance of acquired characters” into the flow of modern biology principles and in this way to offer a logic explanation for transforming internal and external environmental factors into elements that can be transmitted across the generations (Gavrilă et al., 1994; Gavrilă, 1995, 2005).

The Lamarckian principle of inheritance of acquired characters was finally incorporated as such in Darwinian theory of evolution by mean of natural selection, as well as into Modern Synthetic Theory of Evolution as it was the case of almost all the theories that at their time appear to be so called non-darwinian way for interpretation of life evolution (e.g. Kimura-Ohta Neutral Theory of Evolution, 1968, Ohno’s theory of evolution by gene
duplication, 1970, Gould’s and Eldridge’s punctuated equilibrium theory of Evolution or Buican’s theory of multipolar selection and synergic evolution proposed in 1989). All these theories were integrated in the general flow of concepts that constitute the Modern Theory of Biological Evolution, whose core is represented by Darwinian Evolution by mean of Natural Selection.

The epigenetical processes represent a basis for biological memory within and across generations. Environmental factors can play a substantial role as inducers of different epigenetic mechanisms, as it was noticed both in simple eukaryotes (e.g. yeasts) and in complex eukaryotes, including humans.

These epigenetic mechanisms include DNA methylation, changes in chromatin architecture conformation, histone chemical modifications and noncoding small RNAs involvement. There is an epigenetic memory which induces an extensive and orderly reprogramming of genome-wide chromatin modification associated with specification and early development of germ cells in mice (Seki et al., 2005; Santos & Dean, 2004) establishing the specificity of so called epigenetic information and reprogramming of genome function through epigenetic inheritance (Rideout et al., 2001), based on gene and genome imprinting, gene DNA methylation, chromatin architecture changes and specific noncoding snRNA that govern changes in DNA methylation and chromatin conformation.

In eukaryotes, complex regulatory networks have been identified during the development of a multicellular organism.

Each differentiated cell type has its own epigenetic profile, which reflects its genotype, developmental history and environmental influences. Some cells undergo major epigenetic reprogramming during fetal development. The proper or improper handling of these highly sensitive periods may have significant effects both on the newborn and on his/her progeny.

A timing of zygotic activation of embryonic transcription which is a species – specific variable was proposed. In many non-viviparous species of Pisces (e.g. *Danio rerio*, *Oryzias latipes*) and Anura (e.g. *Xenopus laevis*), the early ontogenesis is characterized by rapid and synchronous cleavage cell-divisions driven by different maternally synthesized and stored gene products and especially ribonucleoprotein (RNP) particles, that can be seen as extrachromosomal multiple nucleoli tapeting internal membrane of nuclear envelope in Urodèles like *Salamandra salamandra* and *Triturus cristatus* (Steopoe et al., 1985; Burlibaşa et al., 2005, 2008, Burlibaşa, 2006).

Environmental Epigenetics and Pathogenesis

In the last years a major body of information underlining the complex interactions between gene and environment via epigenetic regulation, was accumulated.

In a recent paper, R.L. Miller and Shuk-Mei Ho (2008) stipulated that findings from multiple epidemiologic and experimental studies indicate that asthma risk may be modified by an epigenetic regulation. *In vitro* data indicate that DNA methylation of genes critical to T-helper cell differentiation may induce polarization toward or away from an allergic phenotype, depending on influence of timing exposure, dose exposure, diet and ethnicity on susceptibility to asthma development. Refering to asthma, the authors express the hope that “the study of environmental epigenetics will help us understand a theoretically preventable environmental disease”.

Some prospective studies indicate an association of prenatal exposure to environmental tobacco smoking (ETS) with impaired respiratory function (fig. 1), transient
wheeze, asthma and/or respiratory infections in infants, young children and teenagers (Magnusson et al., 2005; Alati et al., 2006).

In addition to ETS, low maternal intake of foods containing vitamin E and zinc, or the use of antibiotics during pregnancy can increase the risk for childhood asthma, while maternal intake of probiotics, and higher levels of fruits, vegetables, and oily fish during pregnancy may decrease the risk for this pathological condition in childhood (Devereux et al., 2006; Jedrychowski et al., 2006; Kukkonen et al., 2007; Fitzsimon et al., 2007).

Miller and coworkers (2004) reported different respiratory pathologies among children jointly exposed to prenatal polycyclic aromatic hydrocarbons (PAHs) and postnatal ETS, while Hamada and coworkers (2007) have shown that intrauterine exposure to airborne pollutants increased the risk for respiratory disease in offspring. Using mice as animal model, they demonstrated that exposure of pregnant females to residual oil fly ash, led to a greater susceptibility to an asthmalike phenotype in offspring mice including airway hyperresponsiveness and inflammation, such as eosinophil infiltration and goblet cell hyperplasia.

There are data suggesting that the risk of ETS exposure for asthma may be transmitted across two generations. Grandmaternal smoking during the mother’s fetal period may be associated with a greater asthma risk in the grandchildren, independent of maternal smoking. The risk for asthma in grandchildren was heightened if both the grandmother and mother smoked during pregnancy of the later one. Early postnatal environmental exposures modify the risk for developing later childhood or adult-onset asthma.

Very low birth weight and prematurity are two pathology-like conditions that have been associated with prenatal exposure to pollutants such as ETS and PAHs as it was documented by Windham and coworkers (2000) and by Perera and coworkers (2003).
Exposure to adverse environmental conditions during the growth of airways appears to affect the risk for developing asthma at later life stages, including childhood and adulthood (Miller, R.L. & S.M., Ho, 2008).

The asthma was sometimes considered to be determined only by interactions between genetic polymorphisms and environmental exposures, but nowadays epigenetic mechanisms including developmental reprogramming and imprinting are also considered as important factors in inducing this pathology as it was suggested by Tang W. and Ho S.M. (2007).

Infections incurred during pregnancy have been hypothesized to transmit transgenerationally the epigenetic imprints of infections and inflammation, reducing the offspring’s ability to withstand environmental pathogens and in turn, impact later morbidity and mortality (Rahman, 2003; Finch and Crimmins, 2004).

It is documented that exposure to the reactive oxygen species like $\text{H}_2\text{O}_2$ can induce an increase in histone-acetylase (HAT) activity that promotes chromatin acetylation and remodeling chromatin transcription activity (Rahman, 2003).

Fedulov and coworkers (2008) demonstrated that pulmonary exposure to diesel exhaust or titanium dioxide particles during pregnancy causes increased neonatal asthma susceptibility, expressed by a greater airway inflammation and hyperresponsiveness in the offspring.

In a recent study, Olga Naidenko (2009) affirms that there is an environmental instruction for the genome. While every cell in the multicellular animal body has in essence the same genome, there are multiple “epigenomes” in such a body, everyone representing a peculiar pattern of gene activation for every type of differentiated mature cell of such a multicellular organism. There is an epigenetic profile of the organism, a kind of software, telling the computer when and how to do its work. In the life of the animal such software is written by what it is doing, breathing or eating. This software takes the form of methylation tags attached to the top of nucleotide sequence of a gene sequence, having the potential of turning on or off such genes, depending on concrete environmental conditions. Each tag is like a command in a computer program. Some tags are heritable; some can change throughout life.

Naidenko recognized the toxic effects of environmental pollution on human health and underlined that developing fetus is exposed to hundreds of industrial pollutants which find their way from the mother’s body across the placenta, into the umbilical cord blood and then into the growing body of the child. Laboratory experiments, using animal models, suggest that these effects can be dramatic. If a gestant rat female is transiently exposed to vinclozolin (a common vineyard fungicide) or to methoxychlor (an insecticide) both agents having clear endocrine disruptor effects, the male offspring grows up exhibiting lower sperm counts and infertility, as it was observed in a study performed in Center for Reproductive Biology at Washington State University, such adverse changes in reproductive system of male being associated with epigenetic chemical modification.

Despite the fact that the twins have identical nuclear DNA, their epigenetic profile can induce some differences between them particularly when they age. It was concluded that there is an epigenetic code, specific for every organism.

In early life, the influence of dietary and environmental exposures can have a profound effect on the epigenetic code, potentially resulting in diseases later in the life, including the speed of aging and the risk of developing different pathological conditions like respiratory illness, heart disease, neurodegenerative disease, cancer, as well as behavioral and cognitive illnesses.
In the study with vinclozin it was noticed that DNA methylation changes were passed through the germ line, the effects being encountered in four subsequent generations. In addition to reproductive problems, the adult animals developed a number of diseases affecting prostate, kidney, immune system, lipid metabolism, testis and breasts.

It was shown that traffic pollution causes genetic changes in the womb that increase the child’s risk of developing asthma. The “culprit” is a pathological condition appearing as a consequence of prenatal exposure to PAHs created as by-products of incomplete combustion of carbon – containing fuels such as gasoline. When a mother is exposed to PAHs during pregnancy, methylation of specific genes is affected in the developing fetus, associated with four times greater incidence of asthma symptoms in children, prior to age 5. PAH levels are high in the air in heavy – traffic areas, posing health risk for inner – city neighborhoods and residential communities in the proximity to major highways (Hamada et al., 2007).

Shuk-Mei Ho, at the Cincinnati University affirms: “Our data support the concept that environmental exposure can interact with genes during key developmental periods to trigger disease onset later in the life, and the tissues are being reprogrammed to become abnormal later.”

During the 1990s, it came as a surprise that many industrial chemicals can interfere with the endocrine systems of many species, including humans. There is now some consistent evidence that specific of hormone – related changes can be passed from one generation to the next by epigenetic mechanisms (i.e. “second genetic code”). It was documented that stress, smoking and pollution can cause epigenetic changes being involved in the appearance of many diseases including cancer. It was reported that Dutch women who went hungry during World War II gave birth to small babies, who, in turn, gave birth to small babies even though themselves had plenty to eat (De Biasi Deborah, 2006).

In 2006, Michael Skinner at Washington State University in Spokane declared that “environmental pollution could permanently reprogram the genetic traits of a family line of rodents, creating a legacy of sickness... There is a long – term danger from environmental pollution.”

The researchers are looking at the role epigenetics plays in various kinds of cancer, schizophrenia, bipolar disorder, Parkinson’s disease, Alzheimer’s disease, lupus and other illnesses.

Genes seem to play a part in all of these diseases, but not always the starring role. The problem is also connected to epigenetic mechanisms alteration that controls which genes are turned on and off, and how much protein they are coding is produced.

In a number of kinds of cancer, a gene that suppresses tumour growth appears to get turned off. Moshe Szyff (cited by De Biasi Deborah, 2006) a pioneer in the field of epigenetics is working with his colleagues from McGill University in Montreal. Szyff discovered a way to turn this silenced gene to a functional one using one of the two epigenetic cancer drugs now tested in clinical trials by the Montreal company MethylGene. Azacitidine or Vidaza has been approved in the USA, as a new epigenetic cancer drug, having 16% positive results in treatment of certain types of cancer.

Arturas Petronis, at the Centre for Addiction and Mental Health in Toronto is working on the epigenetic profiles of both schizophrenia and bipolar disorder (manic depression), looking for a pattern of on – off switches that is distinctive in these psychotic condition compared to healthy people.

Moshe Szyff affirmed that “Epigenetics will completely change the face of medicine.” It also may change the way we think about pollution, or the chemicals in many products we use every day. These are data suggesting that heavy metals, pesticides, diesel exhaust, tobacco smoke and other chemicals in the environment may interfere with the human on / off gene
switching mechanisms. There is a justified fear that endocrine disrupters (i.e. so–called gender–bender chemicals) may somehow be the very consequence of switching genes on and off, since their experimental use resulted in fish with both male and female sexual organs or male alligators with shrinking penises.

Researchers in Montreal have found that the boys in neighbourhoods with high crime rates who do not get in much trouble tend to have higher level of cortisol than boys join gangs or steal cars. Their higher stress level seems to make them more fearful, and less likely to engage in risky business (De Biasi Deborah, 2006).

**Epigenetics, Nutrigenomics, Nutriepigenomics, Epidemic “Diabesity” and other pathologies in humans**

Nutrigenomics and Nutriepigenomics are two new fields of BioMedicine in a rapid emerging dynamics explaining involvement of gene – diet/drug interaction in DNA methylation and its contribution to complex diseases: from cancer to schizophrenia (Singh et al. 2003).

Robert Waterland (2009) admitted that epigenetic processes are emerging as major factors in obesity, diabetes and heart disease. Early nutrition might play a critical role in disease susceptibility.

Nutrients influence DNA methylation and in this way can induce gene activation/silencing. New technologies, such as methylation-specific amplification microarray that amplifies hypermethylated regions of the genome, will be useful in identifying epigenetic modifications associated with obesity and other disorders.

Using the microarray examination of hypotalamus in newborn and weanling non-agueoti mice, Waterland and coworkers (2009) detected genetic mutations in different genes (i.e. for leptin, melanocortin 4 receptor, neuropeptide Y etc.) involved in hypothalamic regulation of energy balance, all of them leading to obesity. Huge epigenetic changes in the hypothalamus represent an “epigenetic storm” expressed by some four-fold increase or decrease in methylation during 21-day period.

Leibel R. et al. (2009) studying diabesity in humans reached the conclusion that genetic and epigenetic factors render an individual more or less vulnerable to obesity with its multiple social and health consequences.

The obesity and type 2 diabetes are considered complex disorders with strong genetic predispositions. It was underlined that genes predisposing to obesity act through a permissive environment to produce an obese individual.

Tanya Kral considers that both genetic and environmental factors can influence eating trait and weight gain.

The elevated leptin levels identified in arcuate nucleus in a newborn having two-three weeks of life have a trophic effect on the projections from this brain nucleus, including reductions in their length and density. Leptin may act to promote formation of pathways in neural circuits from arcuate nucleus, exclusively during a restricted critical period in the first two weeks of life when both low and high leptin levels may have negative effects (Bouret, 2009).

There are investigations produced the concept that the “early life adversity” can lead to long term epigenetic effects resulting in metabolic syndrome and impairments in brain development and function (Coplan, 2009).

How do the genome and epigenome interact with each other and the environment leading to obesity and other disease and disorders is still an open question.
In a presentation to New York Academy of Sciences, Robert Waterland from Baylor College of Medicine USA Children’s Nutrition Research Center affirmed the following statements:

- “Epigenetic processes are emerging as major factors in obesity, diabetes, and heart disease; these effects are not caused by genetic mutations, but are still maintained as our cells divide. This is a typical case of "acquired characters inheritance" (i.e. a typical Lamarckian principle);
- Even genetically identical animals and humans (twins) can show differences in character, appearance and physiology;
- Early nutrition might play a critical role in disease susceptibility: nutrients influence DNA methylation, thereby they are influencing gene activation and silencing;
- New technologies, such as a methylation – specific amplification microarray that amplifies hypermethylated regions of the genome, will be useful in identifying epigenetic modifications associated with obesity and other disorders, related to nutrition.
- We might think, intuitively, that excess or deficiencies of nutrients could affect the establishment of DNA methylation”.

Disregulation in epigenetic pathways can lead to obesity. This has been documented in cloned mice: when mice are successfully cloned from somatic cells of adult animals, the newborns often are normal weight, but go on to develop adult – onset obesity and diabetes. Although the mechanism by which this occurs is not yet understood, it is clearly an epigenetic phenomenon because the act of cloning does not change the mouse genotype. Therefore, something in the cloning process must induce epigenetic alterations in the cloned animals (Waterland, 2009) and there is certain truth in Waterland’s statement that “the genes aren’t everything”. The agouti A^{Y} mice, ubiquitously express the agouti protein. These animals are hyperphagic and obese because systemic expression of agouti protein impairs the satiety mechanism. Heterozygotic A^{Y}/a mice are hypermethylated at A^{Y} locus and therefore are protected from obesity. It is a clear example of epigenetics affecting obesity (fig. 2).

**Figure 2.** Identical heterozygote (A^{Y}/a) mice sisters exhibiting obvious body size differences. However the one on the left is hypomethylated , while that on the right is hypermethylated at the gene locus A^{Y}. A^{Y} is a Mendelian gene for yellow fur color, with lethal effect when it is in a homozygous conditions, while in a heterozygous one is viable. A^{Y} = Agouti viable yellow mouse (after R.Waterland, 2009).
Waterland compared one group of pregnant mice given a supplement that enhanced methylation to a group that did not receive supplements and noticed that the first one tended to be a bit lighter than the unsupplemented mice.

From this experiment a clear conclusion emerged: there is an interaction among genotype, epigenotype, and diet that influenced weight and adiposity (Waterland, 2009).

Genetic, epigenetic, environmental, and behavioral factors act in concert to determine vulnerability to obesity and its consequences. Epigenetics suggests that nutrition and other environmental factors present during very early development can strongly influence an individual’s susceptibility to diseases such as obesity and diabetes. Predisposing factors passed through the generations for example, diet and pollution – can influence an individual’s health (Liebel et al., 2009).

Having an obese mother during pregnancy and lactation is a double whammy for obesity – prone animals (Levin et al., 2009). Under – or overnutrition in the early postnatal period can lead to permanent changes in the phenotype (Bouret, 2009).

General conclusions:

1. Genetics and Epigenetics collaborate in explaining normal features of all organisms and some inborn errors of metabolism as well as other inherited pathological conditions both in animals and humans;
2. Scientists are rewriting the laws of heredity as they learn more about a mysterious “second genetic code” that turns our genes on and off. “It changes the whole way we think about inheritance” said Anne Mcilroy in 2006;
3. There is a complex interplay between prenatal environmental exposures gene interaction, and the risk for different pathological conditions including asthma risk (Muller and Ho, 2007);
4. Prenatal exposures to environmental pollutants may lead to chronic diseases later in the life, and there is a need to act quickly and decisively to protect the health of our children from toxic environmental exposures (Olga Naidenko, 2009);
5. Some chemical are more harmful than anyone ever suspected (P. Montague, 2006);
6. Environmental pollution could permanently reprogram the genetic traits of a family line of rodents, creating a legacy of sickness (Michael Skinner, 2000);
7. Since there is an epigenetic memory in every cell of our body, there must be a complex interrelationship between epigenetic memory of neurons and their engramma production ability that function in general short-term and long-term memory (Gavrila, 2009);
8. As for our modern lifestyles, exercise is good, but not just for burning calories. It may reprogram our genes (Moshe Szyf, 2006);
9. You inherit DNA, but it doesn’t tell you if you are living in a rich or a poor environment (Michael Skinner, 2006);
10. Epigenetics may revolutionize medicine (Dr. Moshe Szyf, 2006);
11. “We might think, intuitively, that excesses or deficiencies of nutrients could affect the establishment of DNA methylation”, (Robert Waterland – NYAS, 2009);
12. Genetic, epigenetic, environmental and behavioral factors act in concert to determine vulnerability to obesity and its consequences (Leibel et al., 2009).
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