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The Basic Science and Hype of Epigenetics

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Abstract

Knowledge about the science of epigenetics is very less compared to traditional genetics. Genetics is based on inheritance of genes from parents to offspring and cell lineages. Embryo (which is diploid) is formed as result of fusion of haploid male gamete and haploid female and thus starts as a single cell, and ends up as a group of cells. Genetic changes are stable and rarely reversed, whereas epigenetic changes are often reversed. Any change or mutations that occur in a somatic cell, then all its descendents would be expected to have the same genotype. On the other hand epigenetic changes often occur in groups of cells due to a specific signal which can influence a group of cells with the same receptor and are inheritable. X chromosome inactivation is an excellent example. Epigenetic genome imprinting is the change imposed on DNA sequence and may be lost during development. If they persist either erased or reset during gametogenesis. Adverse environment, can effect change in genotype, but there is no inheritance of acquired characteristics. Epigenetics is quite different, because normal development depends on communication between cells. Thus, a hormone, morphogen or growth factor may induce an epigenetic change that may be heritable. It means that the environment of a cell is important in determining its properties or its fate in the developing organism. Hence, epigenetics is considered as part of Lamarckian inheritance. The aim of this article is to discuss the science of epigenetics, inheritance mechanism of epigenetic changes and genome imprinting and the hype created by the observations made in the field of epigenetics.

Keywords: Epigenetics, gene imprinting, gene dosage, epigenotype, X Chromosome inactivation, DNA Methylation, Genome wide association studies, Genetics

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Introduction

Have you ever thought why identical twins are not exactly, identical! We must have noticed that some of our actions do not resemble that of our parents and they are unique to that particular person, why? If you are a genetic researcher and got weird results in your genetic experiments and it doesn't seem to make any sense – then the answer is epigenetics.

Epigenetics is the branch of science with wide range of effects on many aspects of science and enormous potential in human medicine [1]. The study of heritable changes in gene expression that does not involve any changes in the DNA sequence is called epigenetics. In other words epigenetics means, "A change in phenotype of organism without a change in genotype which in turn affects the cell in reading the genes". Epigenetics is one of the burning research topics and challenging many questions to the scientists who are working in the field of genetics. New and ongoing research is continuously uncovering the role of epigenetics in a variety of human disorders and fatal diseases.

Epigenetics History

During mid 20th century, Conrad H. Waddington and Ernst Hadorn are the two scientists who combined genetics and developmental biology and did research. This later was evolved as a different field of genetics called epigenetics. The term epigenetics was coined by Waddington in 1942, derived from the Greek word "epigenesis" which literally means "in addition to changes in genetic sequence". During the 1990s there became a renewed interest in genetic assimilation. This lead to elucidation of the molecular basis of Conrad Waddington's observations in which environmental stress caused genetic assimilation of certain phenotypic characteristics in Drosophila fruit flies. Since then, research efforts have been focused on untangling the epigenetic mechanisms related to similar types of changes [2].

In the sequence of the human genome there are just four bases, yet with cytosine in methylated or non-methylated form, there are five, and there is the possibility of six [3]. The epigenome project sets out to determine the pattern of cytosine methylation in a variety of cell types [4]. The epigenome project will take a long time to complete; nevertheless along the way, we can expect that interesting information will be continually uncovered. We might expect that some regions of the DNA will have the same, or a very similar pattern of methylation in all cell types. These sequences will include many repetitive or transposable elements which have entered the genome at some time and have been silenced by DNA methylation. Much more interesting information will come from specialized genes that are active in one cell type and inactive in another. The importance of DNA methylation in determining the cell phenotype will then be revealed. In the epigenome project, a new terminology will be necessary to classify differences in DNA methylation between cell types. This introduces the concept of the epigenotype. It has been suggested that the epigenotype is the actual pattern of gene activity in a specialized cell type [5]. These cells have enzymes and proteins necessary for normal metabolism in all cell types, and also luxury proteins which have specialized functions. The epigenotype includes all those genes necessary for both household and luxury functions, and also those that are silent or repressed in a given cell type. Thus a muscle cell and lymphocytes have the same genotype, inherited from the same fertilized egg, but they have very different epigenotype [3].

Epigenetics - Basic science

Epigenetics is mainly concerned with the additional information layered on top of the four letter language sequence (A, G, T, and C) that makes up DNA. There are different types of epigenetic mechanisms and each one guides the proteins in the cell to process specific parts of the DNA in specific ways. For instance, DNA can be tagged with methyl groups that bind to some of its C (cytosine) residues. Other molecules that can bind to DNA molecules are called histone proteins [6]. There are proteins that specifically identify and bind to these methylated areas, so that activity of the genes will be turns on and turns off. These methyl groups and other molecular tags can attach to different locations on the histone proteins and each one has a different effect. Some groups in some locations loosen the attachment between the DNA and the histone, making the DNA more accessible to the proteins that are responsible for activating the genes in that region. Other groups in other locations on DNA do the opposite, or attract other proteins with other specific functions. There are epigenetic marks that cluster around the start points of genes; there are marks that cover long stretches of DNA, and others that affect much shorter regions [5].

Even though every cell in our body has the same DNA sequence, a couple of letters in DNA sequence can jumble here and there, this leads to a different type of affect of that particular gene in different cells. For example, a brain cell doesn't need to follow the same parts of the instruction manual as a spleen cell do. Interesting thing about epigenetics is that these genetic changes are not fixed in the same way the DNA sequence is, some of them can change anytime in your life in response to outside influences while some can even be inherited [3, 4, 5].

Types of epigenetic mechanism

Change is a continuous and natural process that occurs over a period of time in all living organisms. Epigenetic change can be influenced by several factors such as age, the environment, lifestyle, many agents including heavy metals, pesticides, CO_2 emission from motor vehicles, radioactivity, bacteria, viruses etc. In the recent years there is increase in the number of research studies which have focused to understand epigenetics and genome wide distribution of changes [7, 8]. New and ongoing research is continuously uncovering the role of epigenetics in a variety of human disorders and fatal diseases.

Epigenetic processes are essential for the survival of many organisms. At the same time epigenetic change can have more damaging effects that can result in adverse health and behavioral effects [7]. Many types of epigenetic processes have been described which includes methylation, acetylation, phosphorylation, ubiquitylation, sumolyation, chromatin remodeling, histone modifications, and non coding RNA mechanisms (ncRNA). Currently, DNA methylation is one of the most broadly studied and well-characterized epigenetic modifications dating back to studies done by Griffith and Mahler in 1969 which suggested that DNA methylation may be important in long term memory function [7, 8].

Epigenetic inheritance

This is an area where the hype was created and spread across the world faster than the actual fact. Of course, there have been some interesting studies on the inheritance of epigenetics. But, most of the evidences seen in epigenetics are from research done on mice only. There have been indications that some of these findings can also be applicable to human inheritance, but we have only just started to unravel this new phenomenon [8].

It is known for some time that certain environmental factors experienced by adult mice can be inherited their offspring via epigenetic mechanisms. For example a gene in brown mice called agouti, which is methylated found in normal mice whereas mice with an unmethylated agouti gene are yellow in colour and found overweight (obese) compared to normal brown mice, despite being genetically similar to their skinny brown relatives. When alteration in the diet to pregnant mice was made, it resulted in altered ratio of brown to yellow offspring. In another study, supplementation of maternal diet with Genistein along with other compounds induced alterations in DNA methylation which were also inherited in next generation resulting in coat color changes. Supplementation of folic acid resulted in more brown pups, while Bisphenol A (BPA) resulted in more yellow pups [7, 8].

Research on the epigenetic inheritance of addictive behavior is less studied, but does look quite promising. Recent studies in rats demonstrated that exposure to a compound called THC (the active compound in cannabis) during adolescence can affect offspring to display signs of predisposition to heroin addiction [9, 10]. There are some interesting evidences of epigenetics in human beings too. An observational study done in Sweden and the Netherlands population, whose ancestors survived through periods of starvation showed that the effects of acute insufficiency of food on epigenetics and health can pass through at least next three generations. Therefore it can be stated that nutrient deprivation in a recent ancestor can prime the body for early onset of diabetes and cardiovascular problems [11, 12].

Epigenetic field is growing quickly and with it the understanding that both the environment and individual lifestyle can also directly interact with the genome to influence epigenetic change. These changes may be seen at various stages throughout a person's life and/or in later generations. For example, human epidemiological studies have provided evidence that prenatal and early postnatal environmental factors influence the adult risk of developing various chronic diseases and behavioral disorders [9, 10, 11]. Studies have shown that children born during the period of the Dutch famine from 1944-1945 have increased rates of coronary heart disease and obesity after maternal exposure to famine during early pregnancy compared to those not exposed to famine [12]. Less DNA methylation of the insulin-like growth factor II (IGF2) gene, a well-characterized epigenetic locus, was found to be associated with this exposure [7, 8]. Likewise, adults that were prenatally exposed to famine conditions have also been reported to have significantly higher incidence of schizophrenia [10].

Inactivation of chromosomes to compensate gene dose

In females there are two X chromosomes (sex chromosomes - XX), while males have only one X chromosome (sex chromosomes - XY). This difference with respect to sex chromosomes between the sexes creates a need for dosage compensation to adjust the gene dose of X linked genes. Therefore, dosage compensation can be accomplished by silencing one of the two X chromosomes in females. This process is referred as X chromosome inactivation [14]. In mouse and human embryos, X chromosome inactivation is initiated in the early stage of embryo development. This process is regulated by a cis-acting master switch locus called X-inactivation center (Xic), which includes ncRNA gene Xist (X inactive specific transcript) and its antisense transcription unit Tsix/Xite (Xist spelled backward due to its antisense orientation to Xist).

The Xic senses the number of X chromosomes and produces the noncoding Xist RNA from one of the two chromosomes to trigger silencing in cis. Therefore, the initiation of this random inactivation presents important questions on how cells count the number of X chromosomes and choose which one to be inactivated. Recent progress in understanding the mechanisms driving the X chromosome inactivation counting and choice has indicated that multiple regulatory systems may be involved, thus giving rise to multiple models for the initiation of random X chromosome inactivation [15]. Among these interesting findings, trans-interaction of X chromosomes via a novel X pairing region of Xic has been observed, suggesting that the homologous pairing may enable a cell to detect the number of X chromosomes and coordinate Xist/Tsix expression to determine the future active and inactive X chromosomes (Xa and Xi, respectively) [16]. Another recent study supports an alternative mechanism, a stochastic model where each X chromosome has an independent probability to initiate the X chromosome inactivation within a limited period. These studies suggest the presence of a novel X-encoded trans-acting activator involved in initiation of X chromosome inactivation, based on observations in tetraploid ES cells [17]. On the other hand in random inactivation, in some mammals the parental origin determines which X chromosome is to be inactivated [14]. All tissues of marsupials and the extra-embryonic tissues of mice display imprinted inactivation of the paternal X chromosome. The molecular basis underlying the preferential paternal X inactivation and the nature of imprints are not currently well understood.

Once the future Xi is chosen, X chromosome inactivation starts with the accumulation of Xist RNA along the Xi. The Xist expression is regulated by the Tsix gene that acts primarily in the nucleus and is transcribed in the antisense direction over the Xist gene [18]. The Xist RNA coating-induced silencing accompanies multiple layers of epigenetic modifications on the Xi, which lock in and stably maintain the inactive state through cell divisions [14, 15]). Chromosome wide studies revealed various X-linked histone modifications, including hypoacetylation of histone H4 [19], trimethylation of H3K9 and H3K27 [20, 21], H4K20 monomethylation [22], H2AK119 monoubiquitylation [23], as well as substitution of core histone H2A with the histone variant macroH2A [24]. In addition to the histone modification profile, the Xa and Xi allele-specific DNA methylation patterns have also been established [25]. Analysis of Dnmt1–/– embryos has shown that methylation is required for stable maintenance of gene silencing on the Xi [26]. As discussed above, a wide range of chromatin modifiers are known to be involved in XCI, including PcG complexes, histone deacetylases, and DNMT [14]. Although the exact combination of histone modifications on the Xi may vary during development and in different lineages and cell types, the order of chromatin modifications leading to X inactivation was postulated based on the observations during female mouse ES cell differentiation [14, 15]. First, Xist RNA transcription and accumulation on the Xi in cis trigger silencing through as yet unknown mechanisms. Then, recruitment of PRC1 and PRC2 mediates H2AK119 monoubiquitylation and H3K27 tri-methylation, respectively.

In the early stages of X chromosome inactivation, the process is reversible and dependent on the presence of Xist RNA. As the cell differentiation takes place, the Xi undergoes deposition of histone macroH2A and histone H4 hypoacetylation, followed by promoter-specific DNA methylation on the Xi. At this phase, the X chromosome inactivation is irreversible and Xist RNA is not required for maintenance of the inactive state. In addition to chromatin modifications on Xi, the Xi shows the shift to late replication during random inactivation [27] and Xist RNA defines a repressive nuclear compartment early on in the X chromosome inactivation process [28]. Thus, the epigenetic marks and temporal/spatial segregation mechanisms contribute to the initiation and maintenance of X chromosome inactivation. Despite significant progress in understanding of molecular mechanisms of X chromosome inactivation, there are still many unanswered questions. For example, the counting and choice process of random inactivation awaits further elucidation of its molecular basis. Similarly, the mechanisms by which Xist RNA triggers recruitment of chromatin-modifying complexes remain unknown. Furthermore, it is still elusive how cis-acting elements and trans-acting factors coordinate and spread silencing across the chromosome [8].

Epigenetics – clinical Examples

Interest in epigenetics has led to new findings about the relationship between epigenetic changes and a host of disorders including various cancers, mental retardation associated disorders, immune

disorders, neuropsychiatric disorders and pediatric disorders.

Any outside stimulus that can be detected by the body has the potential to cause epigenetic modifications. It's not yet clear exactly which exposures affect which epigenetic marks, nor what the mechanisms and downstream effects are, but there are a number of quite well characterized examples, from chemicals to lifestyle factors to lived experiences:

Bisphenol A (BPA) is an additive in some plastics that has been linked to cancer and other diseases and has already been removed from consumer products in some countries. BPA seems to exert its effects through a number of mechanisms, including epigenetic modification [1, 9, 13].

The beneficial effects of exercise have been known for generations, but the mechanisms are still surprisingly hazy. However, there's mounting evidence that changes to the pattern of epigenetic marks in muscle and fatty tissue are involved [8, 9].

Childhood abuse and other forms of early trauma also seem to affect DNA methylation patterns, which may help to explain the poor health that many victims of such abuse face throughout adulthood [3, 9].

Cancer

Cancer was the first disease to be linked to human epigenetics. Studies performed by Feinberg and Vogelstein in 1983, using primary human tumor tissues, found that genes of colorectal cancer cells were substantially hypomethylated compared with normal tissues [1, 13]. DNA hypomethylation can activate oncogenes and initiate chromosome instability, whereas DNA hypermethylation initiates silencing of tumor suppressor genes. An accumulation of genetic and epigenetic errors can transform a normal cell into an invasive or metastatic tumor cell [1, 14]. Additionally, DNA methylation patterns may cause abnormal expression of cancer-associated genes. Global histone modification patterns are also found to correlate with cancers such as prostate, breast, and pancreatic cancer. Subsequently, epigenetic changes can be used as biomarkers for the molecular diagnosis of early cancer [3, 13].

Neurological disorders

Epigenetic changes are also linked to several disorders that result in intellectual disabilities such as ATR-X, Fragile X, Rett, Beckwith-Weidman (BWS), Prader-Willi and Angelman syndromes [2, 14]. For example, the imprint disorders Prader-Willi syndrome and Angelman syndrome, display an abnormal phenotype as a result of the absence of the paternal or maternal copy of a gene, respectively. In these imprint disorders, there is a genetic deletion in chromosome 15 in a majority of patients. The same gene on the corresponding chromosome cannot compensate for the deletion because it has been turned off by methylation, an epigenetic modification. Genetic deletions inherited from the father result in Prader-Willi syndrome, and those inherited from the mother, Angelman syndrome.

Epigenetic errors also play a role in the causation of complex adult psychiatric, autistic, and neurodegenerative disorders. Several reports have associated schizophrenia and mood disorders with DNA rearrangements that include the DNMT genes [10]. DNMT1 is selectively over expressed in gamma-aminobutyric acid (GABA)-ergic interneurons of schizophrenic brains, whereas hypermethylation has been shown to repress expression of Reelin (a protein required for normal neurotransmission, memory formation and synaptic plasticity) in brain tissue from patients with schizophrenia and patients with bipolar illness and psychosis. A role for aberrant methylation mediated by folate levels has been suggested as a factor in Alzheimer's disease; also some preliminary evidence supports a model that incorporates both genetic and epigenetic contributions in the causation of autism [8, 10]. Autism has been linked to the region on chromosome 15 that is responsible for Prader-Willi syndrome and Angelman syndrome. Findings at autopsy of brain tissue from patients with autism have revealed a deficiency in MECP2 expression that appears to account for reduced expression of several relevant genes.

Immunity & Related Disorders

There are several pieces of evidence showing that loss of epigenetic control over complex immune processes contributes to autoimmune disease. Abnormal DNA methylation has been observed in patients with lupus whose T cells exhibit decreased DNA methyltransferase activity and hypomethylated DNA. Disregulation of this pathway apparently leads to overexpression of methylation-sensitive genes

such as the leukocyte function-associated factor (LFA1), which causes lupus-like autoimmunity. Interestingly, LFA1 expression is also required for the development of arthritis, which raises the possibility that altered DNA methylation patterns may contribute to other diseases displaying idiopathic autoimmunity.

Pediatric Syndromes

In addition to epigenetic alterations, specific mutations affecting components of the epigenetic pathway have been identified that are responsible for several syndromes: DNMT3B in ICF (immunodeficiency, centromeric instability and facial anomalies) syndrome, MECP2 in Rett syndrome, ATRX in ATR-X syndrome (a-thalassemia/mental retardation syndrome, X-linked), and DNA repeats in facioscapulohumeral muscular dystrophy. In Rett syndrome, for example, MECP2 encodes a protein that binds to methylated DNA; mutations in this protein cause abnormal gene expression patterns within the first year of life. Girls with Rett syndrome display reduced brain growth, loss of developmental milestones and profound mental disabilities. Similarly, the ATR-X syndrome also includes severe developmental deficiencies due to loss of ATRX, a protein involved in maintaining the condensed, inactive state of DNA. Together, this constellation of clinical pediatric syndromes is associated with alterations in genes and chromosomal regions necessary for proper neurologic and physical development.

Genomic imprinting

Diploid organisms such as mammals carry two copies of autosomal genes, one from each parent. In most cases, both parental alleles have equal potential to be expressed in cells. However, a subset of autosomal genes is subject to genomic imprinting by which the expression is limited to one of the two parental alleles depending on the parent-of-origin of the gene. Genomic imprinting is an epigenetic mechanism conserved in placental mammals, and failure to establish correct imprinting has been shown to cause defects in embryonic and neonatal growth and can result in neurological disorders such as Prader-Willi syndrome [29]. To date about ~80 imprinted genes have been identified in mice, the majority of which are clustered in the genome while there are some solo imprinted genes. Each imprinted gene cluster often encompasses several protein-coding genes over 100–3000 kb DNA, and at least one non-coding RNA (ncRNA) gene [30].

Expression of imprinted genes in each cluster is generally controlled by a single major cis-acting element, the imprinting control region (ICR) [31]. ICRs are CpG-rich DNA sequences that are methylated in only one of the two parental gametes, and thus carry the parental information. This DNA methylation imprint is acquired during gametogenesis. Prior to sex determination, the parental imprints are erased in germ cells formed in the embryonic gonad. As the embryo develops into a male or female, gametic imprints are placed on paternally imprinted genes during sperm production and on maternally imprinted genes during egg formation, respectively. After fertilization, this methylation imprint is maintained on the same parental chromosome through cell divisions. Establishment and maintenance of the imprints require a series of epigenetic machinery. Gametic imprints are established in germ cells by the de novo methyltransferase Dnmt3a [32]. Another member of Dnmt3 family, Dnmt3L, has been shown to be essential for maternal imprinting in female germ cells, whereas its disruption in male germ cells results in meiotic catastrophe caused by retrotransposon reactivation [33, 34]. These imprinted marks are stably propagated through successive cell divisions by maintenance methyltransferase Dnmt1 and its oocyte-specific isoform Dnmt1o [35, 36]. Furthermore, these gametic imprints can be erased in germ lines during genome-wide reprogramming by an unknown demethylation mechanism(s). Although DNA methylation is the most important mechanism for imprinting, it does not appear to be the only mechanism. Histone modification by a mouse PcG protein Eed has been demonstrated to affect a few paternally repressed genes; however, it has a relatively minor effect compared to that of DNA methylation and may only contribute to maintenance of imprints [37]. Similarly, the absence of histone methyltransferase G9a has been shown to exert pronounced effects on paternal repression of placenta-specific imprinted genes [38].

As mentioned above, each imprinted gene cluster contains at least one ncRNA gene that plays a crucial role in silencing of the multiple protein-coding genes in the cluster by cis-acting mechanisms. Despite the differences in gene organization and ICR functions in different clusters, a few common features of imprinted gene expression/silencing can be derived. The unmethylated ICRs are implicated in all six clusters as positive regulators of ncRNA expression. In maternally imprinted clusters (Igf2r/Air and IC2/Kcnq1), the unmethylated ICR

works as a promoter for a paternally expressed ncRNA that is an antisense orientation to at least one of the genes in the cluster. While deletion of the methylated maternal ICR has no effect on maternally inherited alleles, deletion of the unmethylated paternal ICR reverses the parental-specific expression pattern such that ncRNA expression is lost and biallelic gene expression is obtained by abrogation of paternal silencing. Truncation of ncRNAs at these loci also has similar effects on paternal gene expression, relieving silencing of the paternally inherited alleles [39]. ICRs in paternally imprinted clusters appear to utilize different mechanisms to control the imprinted gene expression. For example, the H19 ncRNA at the Igf2/H19 locus is expressed from the unmethylated maternal chromosome but the ICR does not act as a promoter. Rather, it serves as a boundary element for CTCF (CCCTC binding factor) that is a chromatin insulator protein [40]. The CTCF protein binds the unmethylated maternal ICR blocking the interaction of downstream enhancers with Igf2 and Ins promoters, while it does not affect the interaction between the enhancers and H19 ncRNA promoter. On the paternal chromosome, the DNA methylation imprint prevents CTCF binding, thus allowing the enhancers to drive the expression of Igf2 and Ins genes [41]. Interestingly, the CTCF protein has been shown to have an additional function at the Igf2/H19 locus, protecting the maternal allele from methylation post-fertilization [42].

Although there is an obvious involvement of ncRNAs in imprinted gene silencing, it is unclear how they can repress even non-overlapped genes that are several hundred kilobase pairs apart from either side of the imprinted ncRNA gene. The major question on this issue is to determine whether imprinted ncRNAs silence genes through the transcript itself or through the action of transcription. Several models have recently been reviewed to address this question [43]. Given the similarities in silencing mechanisms between genomic imprinting and X-chromosome inactivation many useful insights into imprinting mechanisms may be obtained by examining whether what is known about X-chromosome inactivation can be applied to genomic imprinting. Another important question that remains unanswered is how the gametic methylation machinery distinguishes parental-specific alleles and establishes DNA methylation marks at different regions at different loci.

The increased knowledge and technologies in epigenetics over the last ten years allow us to better understand the interplay between epigenetic change, gene regulation, and human diseases, and will lead to the development of new approaches for molecular diagnosis and targeted treatments across the clinical spectrum.

Conclusion

Epigenetics is the field that is guaranteed to keep generating dazzling headlines and catches the public interest. One interesting direction is the application of high throughput sequencing technologies to the characterization of hundreds of epigenomes. The goal of International Human Epigenomics Consortium (IHEC) is to generate at least 1,000 publicly available ‘reference’ epigenomes from various normal and diseased cell types. The apparent ability of epigenetics to fill some pretty diverse gaps in our understanding of human health and disease, and to provide scientific mechanisms for so many of our lived experiences, makes it very compelling, but we do need to be careful not to over-interpret the evidence we’ve collected so far.

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