POTENTIAL MEDICAL APPLICATIONS OF EPIGENETICS: AN OVERVIEW

Satyanand Tyagi[1]*, Raghvendra[2], Vivekanand Tyagi[3]
[1] Department of Pharmaceutical Chemistry, K.N.G.D Modi Institute of Pharmaceutical Education & Research, Modinagar, Ghaziabad (dt), U.P, India-201204.
[2] Department of Pharmaceutics, Aligarh College of Pharmacy, Aligarh (dt), U.P, India-202001.
[3] Department of Pharmacy (Hons.), Birla Institute of Technology, Pilani (dt), Rajasthan, India-333031
*Email: sntyagi9@yahoo.com

ABSRTACT

The term epigenetics is defined as "the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being". Epigenetics appears in the literature as far back as the mid 19th century, although the conceptual origins date back to Aristotle (384-322 BC). He believed in epigenesis: the development of individual organic form from the unformed. This controversial view was the main argument against our having developed from miniscule fully-formed bodies. Even today the extent to which we are pre-programmed versus environmentally shaped awaits universal consensus. The field of epigenetics has emerged to bridge the gap between nature and nurture. In the 21st century the most commonly find epigenetics defined as 'the study of heritable changes in genome function that occur without a change in DNA sequence. The aim of present article is to provide in depth knowledge about potential medical applications of epigenetics. This article presents a brief review of Epigenetics with an emphasis on its potential applications from medical point of view.

Keywords: epigenetics, epigenesis, DNA-methylation, chromatin remodelling

INTRODUCTON

What is epigenetics?

The human body consists of more than 200 different cell types. All cells contain the same genetic information – the same DNA sequence – and they are therefore, in theory, able to synthesise the same proteins. However, each cell type synthesises a specific set of proteins. Nerve cells synthesise proteins that are necessary for generating nerve cells, muscle cells synthesise proteins necessary for building up muscle fibres, etc. This is what makes each cell type unique. The differences in the protein synthesis result from the activation of some genes and inactivation of others. This specialisation already takes place during early embryonic development and continues throughout life. But how do the cells exercise control over their own development? To a great extent the answer lies in a mechanism for regulation of gene activity called "epigenetic regulation". Epigenetic regulation is a question of "opening" the DNA structure and thereby activating the gene – or inactivating the gene by "closing" the DNA structure. This may sound simple but in reality the cell has to condense two meters of DNA inside its 1/100 millimetres-in-diameter body! In the condensation process, the cell also decides which genes are going to be activated, and which are not – and here a special group of proteins, the histones, plays a central part.

In biology, and specifically genetics, epigenetics is the study of inherited changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence, hence the name *epi-* (Greek: *επί-* over, above) -*genetics*. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism[1]; instead, non-genetic factors cause the organism's genes to behave differently.

The best example of epigenetic changes in eukaryotic biology is the process of cellular differentiation. During morphogenesis, totipotent cells become the various pluripotent cell lines of the embryo which in turn become fully differentiated cells. In other words, a single fertilized egg cell – thezygote – changes into the many cell types including neurons, muscle cells, epithelium, blood vessels etc. as it continues to divide. It does so by activating some genes while inhibiting others[2].

Epigenetics mechanism

Epigenetics was coined by C. H. Waddington in 1942 as a portmanteau of the words *genetics* and *epigenesis*[3]. *Epigenesis* is an old word which has more recently been used to describe the differentiation of cells from their initial totipotent state in embryonic development.

When Waddington coined the term the physical nature of genes and their role in heredity was not known; he used it as a conceptual model of how genes might interact with their surroundings to produce a phenotype. Robin Holliday defined epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms[4]". Thus *epigenetic* can be used to describe anything other than DNA sequence that influences the development of an organism.

The modern usage of the word in scientific discourse is more narrow, referring to heritable traits that do not involve changes to the underlying DNA sequence. The Greek prefix *epi-* in*epigenetics* implies features that are "on top of" or "in addition to" genetics; thus *epigenetic* traits exist on top of or in addition to the

traditional molecular basis for inheritance. The similarity of the word to "genetics" has generated many parallel usages. The "epigenome" is a parallel to the word "genome," and refers to the overall epigenetic state of a cell. The phrase "genetic code" has also been adapted—the "epigenetic code" has been used to describe the set of epigenetic features that create different phenotypes in different cells. Taken to its extreme, the "epigenetic code" could represent the total state of the cell, with the position of each molecule accounted for; more typically, the term is used in reference to systematic efforts to measure specific, relevant forms of epigenetic information such as the histone code or DNA methylation patterns.

Epigenetic was also used by the psychologist Erik Erikson in his psychosocial development theory. That usage, however, is of primarily historical interest. Two predominant epigenetic mechanisms are DNA methylation and histone modification.

Molecular basis of epigenetic

The molecular basis of epigenetics is complex. It involves modifications of the activation of certain genes, but not the basic structure of DNA. Additionally, the chromatin proteins associated with DNA may be activated or silenced. This accounts for why the differentiated cells in a multi-cellular organism express only the genes that are necessary for their own activity. Epigenetic changes are preserved when cells divide. Most epigenetic changes only occur within the course of one individual organism's lifetime, but, if a mutation in the DNA has been caused, some epigenetic changes are inherited from one generation to the next[5].This raises the question of whether or not epigenetic changes in an organism can alter the basic structure of its DNA, a form of Lamarckism.

Specific epigenetic processes include paramutation, book marking, imprinting, gene silencing, X chromosome inactivation, position effect, reprogramming, transvection, maternal effects, the progress of carcinogenesis, many effects of teratogens, regulation of histone modifications and heterochromatin, and technical limitations affecting parthenogenesis and cloning.

Epigenetic research uses a wide range of molecular biologic techniques to further our understanding of epigenetic phenomena, including chromatin immunoprecipitation (together with its large-scale variants ChIP-on-chip and ChIP-seq), fluorescent in situ hybridization, methylation-sensitive restriction enzymes, DNA adenine methyltransferase identification (DamID) and bisulfite sequencing. Furthermore, the use of bioinformatics methods is playing an increasing role. (computational epigenetics).

Mechanism

Several types of epigenetic inheritance systems may play a role in what has become known as cell memory[6]:

Because the phenotype of a cell or individual is affected by which of its genes are transcribed, heritable transcription states can give rise to epigenetic effects. There are several layers of regulation of gene expression. One way that genes are regulated is through the remodeling of chromatin. Chromatin is the complex of DNA and the histone proteins with which it associates. Histone proteins are little spheres that DNA wraps around. If the way that DNA is wrapped around the histones changes, gene expression can change as well. Chromatin remodeling is accomplished through two main mechanisms:

1. The first way is post translational modification of the amino acids that make up histone proteins. Histone proteins are made up of long chains of amino acids. If the amino acids that are in the chain are changed, the shape of the histone sphere might be modified. DNA is not completely unwound during replication. It is possible, then, that the modified histones may be carried into each new copy of the DNA. Once there, these histones may act as templates, initiating the surrounding new histones to be shaped in the new manner. By altering the shape of the histones around it, these modified histones would ensure that a differentiated cell would stay differentiated, and not convert back into being a stem cell.

2. The second way is the addition of methyl groups to the DNA, mostly at CpG sites, to convert cytosine to 5-methylcytosine. 5-Methylcytosine performs much like a regular cytosine, pairing up with a guanine. However, some areas of genome are methylated more heavily than others and highly methylated areas tend to be less transcriptionally active, through a mechanism not fully understood. Methylation of cytosines can also persist from the germ line of one of the parents into the zygote, marking the chromosome as being inherited from this parent (genetic imprinting).

The way that the cells stay differentiated in the case of DNA methylation is clearer to us than it is in the case of histone shape. Basically, certain enzymes (such as DNMT1) have a higher affinity for the methylated cytosine. If this enzyme reaches a "hemi methylated" portion of DNA (where methyl cytosine is in only one of the two DNA strands) the enzyme will methylate the other half.

Although histone modifications occur throughout the entire sequence, the unstructured N-termini of histones (called histone tails) are particularly highly modified. These modifications include acetylation, methylation, ubiquitylation, phosphorylation and sumoylation.
Acetylation is the most highly studied of these modifications. For example, acetylation of the K14 and K9 lysines of the tail of histone H3 by histone acetyltransferase enzymes (HATs) is generally correlated with transcriptional competence.

One mode of thinking is that this tendency of acetylation to be associated with "active" transcription is biophysical in nature. Because it normally has positively charged nitrogen at its end, lysine can bind the negatively charged phosphates of the DNA backbone and prevent them from repelling each other. The acetylation event converts the positively charged amine group on the side chain into a neutral amide linkage. This removes the positive charge, causing the DNA to repel itself. When this occurs,

complexes like SWI/SNF and other transcriptional factors can bind to the DNA, thus opening it up and exposing it to enzymes like RNA polymerase so transcription of the gene can occur. In addition, the positively charged tails of histone proteins from one nucleosome may interact with the histone proteins on a neighbouring nucleosome, causing them to pack closely. Lysine acetylation may interfere with these interactions, causing the chromatin structure to open up.

Lysine acetylation may also act as a beacon to recruit other activating chromatin modifying enzymes (and basal transcription machinery as well). Indeed, the bromodomain — a protein segment (domain) that specifically binds acetyl-lysine — is found in many enzymes that help activate transcription, including the SWI/SNF complex (on the protein polybromo). It may be that acetylation acts in this and the previous way to aid in transcriptional activation.

The idea that modifications act as docking modules for related factors is borne out by histone methylation as well. Methylation of lysine 9 of histone H3 has long been associated with constitutively transcriptionally silent chromatin (constitutive heterochromatin). It has been determined that a chromodomain (a domain that specifically binds methyl-lysine) in the transcriptionally repressive protein HP1 recruits HP1 to K9 methylated regions. One example that seems to refute this biophysical model for acetylation is that tri-methylation of histone H3 at lysine 4 is strongly associated with (and required for full) transcriptional activation. Tri-methylation in this case would introduce a fixed positive charge on the tail.

It should be emphasized that differing histone modifications are likely to function in differing ways; acetylation at one position is likely to function differently than acetylation at another position. Also, multiple modifications may occur at the same time, and these modifications may work together to change the behaviour of the nucleosome. The idea that multiple dynamic modifications regulate gene transcription in a systematic and reproducible way is called the histone code.

DNA methylation frequently occurs in repeated sequences, and helps to suppress the expression and mobility of 'transposable elements': Because 5-methylcytosine is chemically very similar to thymidine, CpG sites are frequently mutated and become rare in the genome, except at CpG islands where they remain unmethylated. Epigenetic changes of this type thus have the potential to direct increased frequencies of permanent genetic mutation. DNA methylation patterns are known to be established and modified in response to environmental factors by a complex interplay of at least three independent DNA methyltransferases, DNMT1, DNMT3A and DNMT3B, the loss of any of which is lethal in mice[7]. DNMT1 is the most abundant methyltransferase in somatic cells[8], localizes to replication foci[9], has a 10–40-fold preference for hemimethylated DNA and interacts with the proliferating cell nuclear antigen (PCNA)[10]. By preferentially modifying hemimethylated DNA, DNMT1 transfers patterns of methylation to a newly synthesized strand after DNA replication, and therefore is often referred to as the 'maintenance' methyltransferase[11]. DNMT1 is essential for proper embryonic development, imprinting and X-inactivation[12].

Chromosomal regions can adopt stable and heritable alternative states resulting in bistable gene expression without changes to the DNA sequence. Epigenetic control is often associated with alternative covalent modifications of histones[13]. The stability and heritability of states of larger chromosomal regions are often thought to involve positive feedback where modified nucleosomes recruit enzymes that similarly modify nearby nucleosomes.

Because DNA methylation and chromatin remodeling play such a central role in many types of epigenic inheritance, the word "epigenetics" is sometimes used as a synonym for these processes. However, this can be misleading. Chromatin remodeling is not always inherited, and not all epigenetic inheritance involves chromatin remodeling[14].

It has been suggested that the histone code could be mediated by the effect of small RNAs. The recent discovery and characterization of a vast array of small (21- to 26-nt), non-coding RNAs suggests that there is an RNA component, possibly involved in epigenetic gene regulation. Small interfering RNAs can modulate transcriptional gene expression via epigenetic modulation of targeted promoters.

RNA transcripts and their encoded proteins

Sometimes a gene, after being turned on, transcribes a product that (either directly or indirectly) maintains the activity of that gene. For example, Hnf4 and MyoD enhance the transcription of many liver- and muscle-specific genes, respectively, including their own, through the transcription factor activity of the proteins they encode. RNA signalling includes differential recruitment of a hierarchy of generic chromatin modifying complexes and DNA methyltransferases to specific loci by RNAs during differentiation and development. Other epigenetic changes are mediated by the production of different splice forms of RNA, or by formation of double-stranded RNA (RNAi). Descendants of the cell in which the gene was turned on will inherit this activity, even if the original stimulus for gene-activation is no longer present. These genes are most often turned on or off by signal transduction, although in some systems where syncytia or gap junctions are important, RNA may spread directly to other cells or nuclei by diffusion.

A large amount of RNA and protein is contributed to the zygote by the mother during oogenesis or via nurse cells, resulting in maternal effect phenotypes.

Prions

Prions are infectious forms of proteins. Proteins generally fold into discrete units which perform distinct cellular functions, but some proteins are also capable of forming an infectious conformational state known as a prion. Although often viewed in the context of infectious disease, prions are more loosely defined by their ability to catalytically convert other native state versions of the same protein to an infectious conformational state. It is in

this latter sense that they can be viewed as epigenetic agents capable of inducing a phenotypic change without a modification of the genome[15].

Fungal prions are considered epigenetic because the infectious phenotype caused by the prion can be inherited without modification of the genome. PSI+ and URE3 discovered in yeastin 1965 and 1971, are the two best studied of this type of prion[16, 17]. Prions can have a phenotypic effect through the sequestration of protein in aggregates, thereby reducing that protein's activity. In PSI+ cells, the loss of the Sup35 protein (which is involved in termination of translation) causes ribosomes to have a higher rate of read-through of stop codons, an effect which results in suppression of nonsense mutations in other genes[18]. The ability of Sup35 to form prions may be a conserved trait. It could confer an adaptive advantage by giving cells the ability to switch into a PSI+ state and express dormant genetic features normally terminated by premature stop codon mutations[19,20].

Functions and consequences

Development

Somatic epigenetic inheritance, particularly through DNA methylation and chromatin remodeling, is very important in the development of multicellular eukaryotic organisms. The genome sequence is static (with some notable exceptions), but cells differentiate in many different types, which perform different functions, and respond differently to the environment and intercellular signalling. Thus, as individuals develop, morphogens activate or silence genes in an epigenetically heritable fashion, giving cells a "memory". In mammals, most cells terminally differentiate, with only stem cells retaining the ability to differentiate into several cell types ("totipotency" and "multipotency"). In mammals, some stem cells continue producing new differentiated cells throughout life, but mammals are not able to respond to loss of some tissues, for example, the inability to regenerate limbs, which some other animals are capable of. Unlike animals, plant cells do not terminally differentiate, remaining totipotent with the ability to give rise to a new individual plant. While plants do utilise many of the same epigenetic mechanisms as animals, such as chromatin remodeling, it has been hypothesised that plant cells do not have "memories", resetting their gene expression patterns at each cell division using positional information from the environment and surrounding cells to determine their fate[21].

Medicine

Epigenetics has many and varied potential medical applications. Congenital genetic disease is well understood, and it is also clear that epigenetics can play a role, for example, in the case of Angelman syndrome and Prader-Willi syndrome. These are normal genetic diseases caused by gene deletions or inactivation of the genes, but are unusually common because individuals are essentially hemizygous because of genomic imprinting, and therefore a single gene knock out is sufficient to cause the disease, where most cases would require both copies to be knocked out.

Evolution

Although epigenetics in multicellular organisms is generally thought to be a mechanism involved in differentiation, with epigenetic patterns "reset" when organisms reproduce, there have been some observations of transgenerational epigenetic inheritance (e.g., the phenomenon of paramutation observed in maize). Although most of these multigenerational epigenetic traits are gradually lost over several generations, the possibility remains that multigenerational epigenetics could be another aspect to evolution and adaptation. These effects may require enhancements to the standard conceptual framework of the modern evolutionary synthesis.

Epigenetic features may play a role in short-term adaptation of species by allowing for reversible phenotype variability. The modification of epigenetic features associated with a region of DNA allows organisms, on a multigenerational time scale, to switch between phenotypes that express and repress that particular gene[22]. When the DNA sequence of the region is not mutated, this change is reversible. It has also been speculated that organisms may take advantage of differential mutation rates associated with epigenetic features to control the mutation rates of particular genes.

Epigenetic changes have also been observed to occur in response to environmental exposure—for example, mice given some dietary supplements have epigenetic changes affecting expression of the agouti gene, which affects their fur colour, weight, and propensity to develop cancer[23, 24].

Cancer and developmental abnormalities

A variety of compounds are considered as epigenetic carcinogens—they result in an increased incidence of tumours, but they do not show mutagen activity (toxic compounds or pathogens that cause tumours incident to increased regeneration should also be excluded). Examples include diethylstilbestrol, arsenite, hexachlorobenzene, and nickel compounds.

Many teratogens exert specific effects on the fetus by epigenetic mechanisms[25]. While epigenetic effects may preserve the effect of a teratogen such as diethylstilbestrol throughout the life of an affected child, the possibility of birth defects resulting from exposure of fathers or in second and succeeding generations of offspring has generally been rejected on theoretical grounds and for lack of evidence[26]. However, a range of male-mediated abnormalities have been demonstrated, and more are likely to exist[27].

Twin studies

Recent studies involving both dizygotic and monozygotic twins have produced some evidence of epigenetic influence in humans[28, 29].

REFERENCES

1. Adrian Bird, "Perceptions of epigenetics", Nature, 447, 2007, (7143): 396–398.

2. Reik Wolf, "Stability and flexibility of epigenetic gene regulation in mammalian development", Nature, 447, 2007, 425–432.

3. C.H. Waddington, "The epigenotype", Endeavour, 1, 1977, 18–20.

4. Holliday R, Mechanisms for the control of gene activity during development, Biol. Rev. Cambr. Philos. Soc., 65, 1990, 431-471.

5. V.L. Chandler, "Paramutation: From Maize to Mice", Cell, 128 (4), 2007, 641–645.

6. Jablonka E, Lamb MJ, Lachmann M, "Evidence, mechanisms and models for the inheritance of acquired characteristics", J. Theoret. Biol, 158 (2), 1992, 245–268.

7. Li E, Bestor TH, Jaenisch R, "Targeted mutation of the DNA methyltransferase gene results in embryonic lethality", Cell, 69 (6), 1992, 915–926.

8. Robertson KD, Uzyolgi E, Lian G, et al, "The human DNA methyltransferases (DNMTs) 1, 3a, 3b: Coordinate mRNA expression in normal tissues and over expression in tumours", Nucleic Acids Res, 27 (11), 1999, 2291–2298.

9. Leonhardt H, Page AW, Weier HU, Bestor TH, "A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei", Cell, 71 (5), 1992, 865–873.

10. Chuang LS, Ian HI, Koh TW, et al, "Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1", Science, 277 (5334), 1997, 1996–2000.

11. Robertson KD, Wolffe AP, "DNA methylation in health and disease", Nat Rev Genet, 1 (1), 2000, 11–19.

12. Li E, Beard C, Jaenisch R, "Role for DNA methylation in genomic imprinting", Nature 366 (6453), 1993, 362–365.

13. Rosenfeld, Jeffrey A, Wang Zhibin, Schones Dustin, et al, "Determination of enriched histone modifications in non-genic portions of the human genome.", BMC Genomics, 10 (143), 2009, 143.

14. Mark Ptashne, On the use of the word 'epigenetic', Current Biology, 17(7), 2007, 233-236.

15. A. Yool and W.J. Edmunds, "Epigenetic inheritance and prions", Journal of Evolutionary Biology, 11, 1998, 241–242.

16. B.S. Cox, "[PSI], a cytoplasmic suppressor of super-suppression in yeast", Heredity, 20, 1965, 505–521.

17. F. Lacroute, "Non-Mendelian mutation allowing ureidosuccinic acid uptake in yeast", Journal of Bacteriology, 106 (2), 1971, 519–522.

18. S.W. Liebman, F. Sherman, "Extrachromosomal psi+ determinant suppresses nonsense mutations in yeast", Journal of Bacteriology, 139 (3), 1979, 1068–1071.

19. H.L. True, S.L. Lindquist, "A yeast prion provides a mechanism for genetic variation and phenotypic diversity", Nature, 407 (6803), 2000, 477–483.

20. J. Shorter, S. Lindquist, "Prions as adaptive conduits of memory and inheritance", Nature Reviews Genetics, 6 (6), 2005, 435–450.

21. Silvia Costa, Peter Shaw, 'Open Minded' cells: how cells can change fate, Trends in Cell Biology, 17(3), 2006, 101-106.

22. O.J. Rando, K.J. Verstrepen, "Timescales of Genetic and Epigenetic Inheritance", Cell 128 (4), 2007, 655–668.

23. Cooney CA, Dave AA, Wolff GL, "Maternal Methyl Supplements in Mice Affect Epigenetic Variation and DNA Methylation of Offspring", Journal of Nutrition, 132 (8 Suppl), 2002, 2393S–2400S.

24. Waterland RA, Jirtle RL, "Transposable elements: Targets for early nutritional effects on epigenetic gene regulation", Molecular and Cellular Biology, 23 (15), 2003, 5293–5300.

25. Gurvich N, Berman MG, Wittner BS, et al, "Association of valproate-induced teratogenesis with histone deacetylase inhibition in vivo", FASEB J, 19 (9), 2004, 1166–1168.

26. Smithells D, "Does thalidomide cause second generation birth defects?", Drug Saf, 19 (5), 1998, 339–341.

27. Friedler G, "Paternal exposures: impact on reproductive and developmental outcome. An overview", Pharmacol Biochem Behav, 55 (4), 1996, 691–700.

28. Kaminsky, Zachary A, Tang, T, et al, "DNA methylation profiles in monozygotic and dizygotic twins", Nature Genetics, 41 (2), 2009, 240.

29. Fraga M. F, "Epigenetic differences arise during the lifetime of monozygotic twins", Proceedings of the National Academy of Sciences, 102, 2005, 10604.

****