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THERAPEUTIC EPIGENETICS – A BOON TO THE FUTURE?

D. Benet Bosco Dhas, *Scientist*
Central Inter-Disciplinary Research Facility

Sri Balaji Vidyapeeth Mahatma Gandhi Medical College and Research Institute Campus
Pillaiyarkuppam, Puducherry - 607403, India
Email: benetbiotech@gmail.com

Abstract ▶ Successful completion of the Human Genome Project gave the hope for development of novel therapeutics, diagnostics for the welfare of humankind. Individual genetic studies and genome wide association studies revealed the genetic risk factors for various diseases which can be used in predetermination. This eventually led to the growth of pharmacogenomics that confers individual drug dosage adjustment preventing from adverse effects. However, it addresses only the hitches raised by the underlying genetic sequence but not external factors that influences the genotypic and phenotypic expression. Epigenetic research deals with these factors and studies the modifications caused along with their phenotype. These modifications are reversible which can be used as target for therapeutics, thus improving the treatment strategies of various diseases. In this review, we attempt to discuss the use of epigenetic modifications as drug targets and their mechanism of action.

Key Words: DNA methylation, DNMT inhibitors, HDACi, HATi, miRNA

Introduction

For the past two decades, genomics was ruling the medical research, deciphering disease pathophysiology, risk factors, prognostic strategies and much more. But still, it could not answer several questions raised by the research community like the influence of environmental stress in differential gene expression. This eventually led to the development of "Epigenetics", which explains the genetic behavior apart from underlying nucleotide sequence. By definition, epigenetics is the study of factors (chemicals, proteins, environmental stress, etc.) that influence differential gene expression in cells without changing the nucleotide sequence.

The journey of epigenetics started less than a century before, when C. H. Waddington coined the term in 1942[1]. Epigenetics always answers a scientific question in three different contexts, DNA methylation, histone modifications and influence of micro RNA (miRNA). A simple example for epigenetic changes is the process of cellular differentiation in a eukaryotic system[2]. Epigenetics was found to play important roles in disease pathogenesis[3], drug resistance[4] and prognosis/diagnosis[5]. Apart from the somatic heritable nature of epigenetic changes, the property that motivate researchers is that these changes are reversible, which led to the development of novel therapeutics [6].

The use of any drug or factors that influence epigenetic changes and benefits the medical treatment is broadly known as "Epigenetic therapy". In a similar definition, the drugs that alters or reverses the underlying epigenetic changes in a diseased conditions are also included in epigenetic therapy. In this review, we attempt to discuss the research conducted

so far, on therapeutic epigenetics, which may have an impact on future medical treatment strategies.

Epigenetics in diseases

One of the epigenetic mechanism, DNA methylation, which was extensively studied, was found to be associated with several diseases such as Rett syndrome[7], diabetes[8], cancer[9] and systemic lupus erythematosus[10]. In most of the diseases, hypomethylation of CpG islands in promoter region of specific genes and decreased DNMT1 (DNA methyl transferase 1), DNMT3B (DNA methyl transferase) expression were observed[11].

Methylation and acetylation are the two important modifications that histones undergo, that led differential gene expression. Histone Acetyl Transferase (HATs) and Histone Deacetylases (HDACs) are involved in histone acetylation, whereas Histone Methyl Transferases (HMTs) and Histone Demethylases (HDMs) influences histone methylation[12]. Acetylation of histones was found to be associated with diseases such as Rubinstein-Taybi syndrome[13], asthma[14], cancer[15] and diabetes[8]. On the other hand, histone methylation was also significantly associated with Sotos syndrome[16], Huntington's disease[13] and cancer[15].

The third epigenetic mechanism, miRNA, influences differential gene expression through complementary binding with coding messenger RNA (mRNA) and subsequent deactivation with the help of Dicer protein and other associated proteins[17]. miRNAs like miR-101[18], miR-143[19], miR-29[20] had decreased expression levels in cancer, whereas expression levels of miR-21[21] and miR-155[22] are found to be increased. The association of miRNA levels was also studied in relation with diabetic conditions (both type I and type II), wherein miR-144[23], miR-146a[24], miR-29[25] and miR-27a[26] are widely demonstrated with promising results.

Pharmacoepigenomics

The successful discoveries made through pharmacogenomics pooled polymorphic allelic data associated with drug response and efficacy under various diseased conditions. For example, cardiovascular patients with *CYP2C19* mutant variants should undergo clopidogrel dose adjustment to get therapeutic effect or to avoid adverse effects[27]. Pharmacoepigenomics emerged as an idea to advance the further understanding of drug response and efficacy through in depth molecular analysis, in the early 1990s.

Pharmacoepigenomics is the study of epigenetic alterations and the factors involved, in relation with drug response in any diseased condition. The first identified pharmacoepigenomic phenomenon was methylation changes in the drug metabolizing enzyme, *CYP2E1*, in relation to birth[28]. Only in the last decade, it was found that tobacco consumption regulates the methylation levels of *CYP1A1* gene promoter[29]. Eventually, several researchers studied the influence of epigenetic changes in drug response in various disease, especially cancer[30]. Recently, promoter hypomethylation in *IGFBP3* was found to be associated with cisplatin response in non-small-cell-lung cancer[31].

Pharmacoepigenomics is also used to predict the outcomes after a chemotherapy. The outcomes of patients with early stage breast cancer after adjuvant tamoxifen therapy can be assessed through *PITX2* promoter methylation[32]. Low recurrence rates of bladder cancer was associated with *CDKN2A* hypermethylation after interleukin-2 therapy[33]. In whole, pharmacoepigenomics can also be applied in the development of novel diagnostic/prognostic markers, predictive markers and therapeutic targets, eventually improving the treatment strategies[34].

DNA methylation as therapeutic target

Global methylation studies showed that DNA methylation (both hyper- and hypo-) have significant roles in disease pathogenesis, progression and outcomes. The most widely studied disease in relation with DNA methylation is cancer. Earlier it was suspected that DNA hypomethylation is the only phenomenon occurring in carcinogenesis[35], but later it was understood that both hypermethylation and hypomethylation of specific genes influences the disease pathophysiology[36]. DNA methylation can be targeted using enzyme inhibitors like 5-azacytdine that binds to DNMTs and prevent further methylation during replication[37].

5-Azacytidine (Vidaza) and its deoxy analogue, 5-aza-2'-deoxycytidine (Dacogen) were approved by the US Food and Drug Administration for the treatment of MDS[38,39]. Treatment with 5-azacytidine improved the survival rate of MDS patients up to 20%[40]. It was also studied in patients with acute myeloid leukemia (AML), whereas the deoxy analogue was studied in chronic myelomonocytic leukemia (CMML) patients[41,42].

A recent epigenome wide association study by Ronn *et al*. revealed altered DNA methylation levels in type 2 diabetes. Some of the genes that are differentially methylated include *TCF7L2, IRS1, PPARG* and *THADA,* involved in pathways of cancer, MAPK signaling and axon guidance[43].

DNA methylation can also be used as potential therapeutic targets in infectious diseases. In our recent study,

significant difference in global methylation was found in newborns with sepsis when compared to non-septic babies[44]. Epigenome wide association studies revealed protocadherin beta gene hypermethylation which was correlated with decreased leukocyte adhesion, a physiological process of neonatal sepsis[45,46].

These epigenetic changes can be targeted with novel drugs, reversing to the original state. For example, curcumin, the natural and edible pigment present in *Curcuma longa* (turmeric) and genistein, another phytochemical compound, showed reversal of hypermethylation of RARβ2 promoter in cervical cancer cell lines[47]. In a mouse model of Alzheimer's disease (AD), the methylating agent, Betaine, was found to improve memory[48].

Histone modifications as epigenetic target

The histone modifications like acetylation and methylation can be reversed by using appropriate enzyme inhibitors. HDAC inhibitors (HDACi) such as Phenylbutyrate and Suberoylanilide hydroxamic acid (SAHA) were used in MDS and AML, improving the hematological parameters[49,50].

Valproic acid (HDACi) was found to be useful in the treatment epilepsy, bipolar disorder[51], cancer[52] and AD[53]. Ricobaraza *et al.,* showed that sodium phenylbutyrate improved memory in AD mouse model[54]. The well-known class III HDACs, also known as Sirtuins (SIRTs), play a role as epigenetic targets in AD and cancer[55].

Inhibition of HAT p300 using C646was found to reduce the acetylated and phosphorylated tau protein levels, *in vitro*[56]. Curcumin also showed HAT inhibiting activity in AD[57].

MicroRNA as epigenetic target

RNA therapeutics targeting the non-coding region of amyloid β precursor protein using erythromycin antibiotic, paroxetine antidepressant and N-acetyl cysteine was found to reduce extracellular amyloid β in AD mouse model[58].

Limitation of therapeutic epigenetics

The target of epigenetic therapy are the genes and pathways affected by the epigenetics mechanisms which triggers a caution of non-specificity. If one attempts to reverse the methylation pattern of a silenced gene (hypermethylated) through some drugs, it may non-specifically effect on other silenced genes like oncogenes. Hence there is an urge to develop technology for gene specific targets for therapeutic epigenetics.

Conclusion

With the extensive bench side knowledge developed through genomic and epigenetic research on disease pathogenesis and progression, its time to implement them bed-side. Development of novel genetic and epigenetic therapeutics will pave betterment of medical treatment strategies.

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