Pharmacology & Therapeutics 173 (2017) 118–134

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B. Teicher

Epigenetics in cancer: Fundamentals and Beyond

Subhankar Biswas, C. Mallikarjuna Rao *

Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, Karnataka, India

ARTICLE INFO

Available online 8 February 2017

Keywords:
Epigenetics
Cancer
Histones
DNA methylation
miRNA
Epigenetic drugs

ABSTRACT

Activation of oncogenes or the deactivation of tumor suppressor genes has long been established as the fundamental mechanism leading towards carcinogenesis. Although this age old axiom is vastly accurate, thorough study over the last 15 years has given us unprecedented information on the involvement of epigenetic in cancer. Various biochemical pathways that are essential towards tumorigenesis are regulated by the epigenetic phenomenons like remodeling of nucleosome by histone modifications, DNA methylation and miRNA mediated targeting of various genes. Moreover the presence of mutations in the genes controlling the epigenetic players has further strengthened the association of epigenetics in cancer. This merger has opened up newer avenues for targeted anti-cancer drug therapy with numerous pharmaceutical industries focusing on expanding their research and development pipeline with epigenetic drugs. The information provided here elaborates the elementary phenomena of the various epigenetic regulators and discusses their alteration associated with the development of cancer. We also highlight the recent developments in epigenetic drugs combining preclinical and clinical data to signify this evolving field in cancer research.

Contents

1. Introduction

Cancer is a group of disease that varies extensively with respect to its origin. As a result, the key hurdles associated with its treatment consist of inappropriate diagnosis leading to recurrences and drug resistance. In relation to this, one of the emerging therapeutic class of antineoplastic agent is aimed at targeting gene expression owing to the fact that irregularities in the expression pattern of a gene is a key feature in the development of cancer (Cavalieri, 1996). To accommodate the entire length of several meters of human DNA in the nucleus of a cell, DNA is coiled around histone proteins forming a complex called nucleosome, the basic unit of chromatin (Annunziato, 2008). In addition, post-translational modifications of histone tail alter the structure of chromatin leading to a change in gene expression which is an element of epigenetic regulation. With our improved understanding about cancer, it is now established that malignant growth is associated with both genetic and epigenetic abnormalities (Sadikovic, Al-Romaih, Squire, & Zielenska, 2008). In particular, epigenetic alterations occur early during neoplastic growth and finally develop into a malignant tumor. Although epigenetic modifications are inherited in somatic cells, yet these modifications are possibly reversible indicating that epigenetic alterations can be a promising therapeutic target to explore. Undoubtedly, the fast growing field of therapeutic epigenetic is being continually expanded by integrating

* Corresponding author.
E-mail address: mallik.rao@manipal.edu (C.M. Rao).

http://dx.doi.org/10.1016/j.pharmthera.2017.02.011
0163-7258/

laboratory results with clinical data suggesting us how epigenetic therapy can be best utilized for the benefit of patient. In this review we take a comprehensive look at the various epigenetic players, their involvement in the development of cancer and the drugs employed in altering those mechanisms.

2. Epigenetic phenomenon

The term Epigenetics was coined by C.H. Waddington in 1942 as "the causal interaction between genes and their products, which bring the phenotype into being". However with our increasing knowledge in molecular biology the definition has evolved as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not involve a change in the sequence of DNA" (Dupont, Armant, & Brenner, 2009). The manner in which chromatin structure is preserved and ordered is crucial in understanding the origin of epigenetic alteration. Certainly, each cell type in the body is genetically identical i.e. they share the same set of genes but needs to differentiate phenotypically into diverse type of cells and tissues to endure a normal functioning human body. This is controlled by highly synchronized regulatory mechanism which involves epigenetics. Epigenetic changes regulate gene expression by hindering the availability of transcription factors towards DNA. These modifications occur at different regions encircling the genome. The fundamental of epigenetic regulation of gene expression takes into account the manner in which DNA is wrapped around nucleosome and also considers the way in which each nucleosome are positioned throughout the genome. With our increased understanding about the biology of cancer attributed by the rapid advances in technology, it is now well established that cancer cell harbor global epigenetic alterations beside various genetic mutations representing a complex interplay between these players (Sadikovic et al., 2008). This phenomenon was evident from gene expression and DNA methylation studies providing the initial clues linking epigenetics with cancer. Emerging data are now strengthening our outlooks of the genome wide role of epigenetics. At present, the most studied epigenetic alterations associated with neoplastic phenotype are variation in DNA methylation, alteration in the structure of histone proteins and gene regulation by small noncoding microRNAs.

2.1. DNA methylation

Adenine, Thymine, Cytosine and Guanine are the key nitrogenous bases which are found in eukaryotic organisms. These bases usually comprise the majority of sequence found in eukaryotic DNA. Apart, from these four major bases, the existence of a fifth base i.e 5-methylcytosine, is one of the major covalent modification of DNA. In eukaryotes, DNA methylation is a common epigenetic alteration and these epigenetic marks are typical of heterochromatin. DNA methylation plays an important role in maintaining the stability of genome, genomic imprinting, inactivation of X-chromosome in females, regulation of transcription and also in the developmental process of an organism (Robertson & Jones, 2000). Methylated DNA is present primarily in repetitive genomic regions (including satellite DNA, like micro and mini-satellites), within centromeres and parasitic elements such as short interspersed transposable elements (SINEs) and long interspersed transposable elements (LINEs) where they function to silence genes and non-coding genomic regions. The 5^{th} carbon of cytosine residues are highly prone to methylation compared to other nitrogenous bases and consist of approximately 1% of the total nucleotides. Moreover, the majority of DNA methylation occurring on cytosine residue is present in the CpG dinucleotide distributed throughout the genome and is also densely found in regions known as CpG islands (Jones & Takai, 2001). These CpG islands overlap the promoter regions of approximately 60-70% of human gene. In normal cells, the promoter regions of genes, especially those preceded by CpG islands are usually unmethylated, allowing transcription factors and other associated proteins to interact with the gene and facilitate their expression. In contrast, the genomes of gametes and cells whose promoter regions are less enriched with CpG islands are frequently methylated during early development. However, we should bear in mind that these genes exhibit a distinct expression control during development and are always tissue specific.

The conversion of cytosine into 5-methyl cytosine (5mC) is carried out by the catalytic activity of a group of enzymes called DNA methyltransferases (DNMTs). These enzymes use S-adenosyl methionine (SAM) as a key methyl group donor which transfers methyl group to cellular elements like DNA, lipids and proteins. SAM is converted into S-adenosyl homocysteine (SAH) after the transfer of methyl group by DNMTs. There are two major categories of the DNMTs in mammalian cells, a maintenance methyltransferase and a *de novo* methyltransferase. The original DNA methylation pattern in a cell is greatly maintained by the catalytic activity of DNMT1, which prefers hemi-methylated DNA in place of non-methylated DNA as a substrate during replication, most likely with the support of UHRF1 (Ubiquitin like with PHD and ring finger domain 1) which also recognizes hemi-methylated sites, suggesting a role in maintaining the methylation patterns during cell division (Qin et al., 2015). In contrast, new DNA methylation pattern are established in the developmental phase of a cell utilizing DNMT3A and DNMT3B, which are expressed all over the cell cycle and shows equal preference for both hemi and unmethylated DNA making them *de novo* methyltransferase. Another enzyme, DNMT3L has been identified which is deficient in the conserved catalytic domain commonly associated with DNA methyltransferase. Although it is accepted that DNA methyltransferase are specific in their functions and non-overlapping, yet recent evidence suggests the overlapping role of *de novo* methyltransferases with maintenance methyltransferase (Walton, Francastel, & Velasco, 2011).

DNA methylation silence gene expression directly by impeding the binding of various transcription factors and indirectly by enrolling methyl-CpG binding domain (MBD) proteins. The MBD family contains five core proteins which include MBD1, MBD2, MBD3, MBD4 and the methyl cytosine binding protein 2 (MECP2). Apart from these, other MBD containing proteins are MBD5/6, SETDB1/2 and BAZ2A/B. The MBD protein employs histone modifying enzymes and chromatin remodeling complexes in methylated sites and facilitates transcriptional repression. Chromatin remodeling complex like NuRD binds with MBD2 protein and methylate DNA (Du, Luu, Stirzaker, & Clark, 2015). These mechanisms play a central role in establishing the critical role of DNA methylation in epigenetic gene regulation.

Although enzymes catalyzing DNA methylation has been well established, recent research has also identified mechanisms involved with the removal of methyl group. The discovery of ten-eleven translocation (TET) [which derives its name based on a recurrent chromosomal translocation t(10;11)(q22;q23)] and activation-induced cytidine deaminase (AID) family of enzymes has provided unprecedented information in our understanding of DNA demethylation (Scourzic, Mouly, & Bernard, 2015). DNA demethylation can be achieved by two processes involving passive and active demethylation. Passive demethylation occurs by the failure of maintenance DNMT enzyme to methylate DNA after replication. Whereas, active DNA demethylation utilizes TET and AID family of enzymes to hydroxylate, oxidize or deaminate 5mC. Three TET family members have been identified so far including TET1, TET2 and TET3 and each of them are involved in distinct cellular process. Hydroxylation of 5mC by TET proteins produces 5-hydroxy methylcytosine (5hmC) and its subsequent conversion into 5-formylcytosine (5-fC) and 5-carboxylcytosine (5caC) followed by deamination and entry into the subsequent base excision repair pathway (Fig. 1) (Zhao & Chen, 2013).

2.2. Histone modifications

The nucleosome core particle which is the basic element of chromatin wraps 147 base pair of DNA around an octamer of four core histone proteins in a 1.7 left handed super helical turn. The inherent positive