Metabolomics: Open Access
ISSN: 2153-0769

Cacabelos, Metabolomics 2014, 4:1
DOI: 10.4172/2153-0769.1000e126

Editorial Open Access

Metabolomics of Epigenetic Drugs: Precautionary Measures

Ramón Cacabelos*

Chair of Genomic Medicine, Camilo José Cela University, Madrid, EuroEspes Biomedical Research Center, Corunna, Spain

Epigenetics involves heritable alterations of gene expression and chromatin organization without changes in DNA sequence. Classical epigenetic mechanisms, including DNA methylation and histone modifications, and regulation by microRNAs (miRNAs), are among the major regulatory elements that control metabolic pathways at the molecular level, with epigenetic modifications regulating gene expression transcriptionally and miRNAs suppressing gene expression post-transcriptionally. Vertebrate genomes undergo epigenetic reprogramming during development and disease. Stable transmission of DNA methylation, transcriptomes and phenotypes from parent to clonal offspring are demonstrated in various asexual species, and clonal genotypes from natural populations show habitat-specific DNA methylation. Methylation varies spatially across the genome with a majority of the methylated sites mapping to intragenic regions. Not only nuclear DNA (nDNA), but also mitochondrial DNA (mtDNA) may be subjected to epigenetic modifications related to disease development, environmental exposure, drug treatment and aging. Epigenetic modifications are reversible and can be potentially targeted by pharmacological and dietary interventions.

DNA methylation is a process by which methyl groups are incorporated into cytosine molecules by DNA methyltransferases (DNMTs), forming 5-methylcytosine and contributing to suppression of transcription. Approximately 70% of CpG dinucleotides within the human genome are methylated. DNA methylation inhibits transcription by interfering with the binding of transcription factors to recognition sites on promoters or by recruiting and binding transcriptional repressors, methyl-CpG-binding proteins (MBDs), and altering chromatin structure into an active state. The incorporation of methyl groups in CpGs is catalyzed by a family of DNA methyltransferases (DNMTs), which in mammals are represented by 2 *de novo* DNMts (DNMT3A, DNMT3B) and a maintenance DNMT (DNMT1) that is expressed in neurons. DNMT2 methylates aspartic acid tRNA, and does not methylate DNA. DNA demethylation can be produced by at least 3 enzyme families: (i) the ten-eleven translocation (TET) family, mediating the conversion of 5mC into 5hmC; (ii) the AID/APOBEC family, acting as mediators of 5mC or 5hmC deamination; and (iii) the VER glycosylase family involved in DNA repair. Reducing the hypermethylation levels in some pathogenic genes may be an alternative therapy in cancer and neurodegeneration. Examples of DNMT inhibitors include (i) nucleoside analogs (5-aza-2'-deoxycytidine (Decitabine), 5-azacytidine (Azacitidine)), (ii) small molecules (hydralazine, procainamide, RG108 [2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl)propanoic acid]), (iii) natural products (curcumin derivatives (RG-108, SGI-1027), psammaplins, tea polyphenols (epigallocatechin-3-gallate), catechins (catechin, epicatechin), bioflavonoids (quercetin, genistein, fisetin)), (iv) antisense oligonucleotide inhibitors (MG98), and (v) ncRNAs (miRNAs). Epigenetic alterations are a hallmark of cancer that govern the silencing of genes. 5-Azacytidine (5-aza-CR, Vidaza) and 5-aza-2'-deoxycytidine (5-aza-dC, Dacogen) are the only FDA approved DNA methyltransferase inhibitors (DNMTi) for the treatment of myelodysplastic syndrome.

Histones are nucleosomic proteins integrated in the nuclear chromatin. Nucleosomes are formed by 147 DNA base pairs wrapped around an octamer of histones, assembled by two copies of each of the four core histones, H2A, H2B, H3 and H4. Histone H1 is the linker binding DNA between the nucleosomal core particles to stabilize chromatin structures. Histones are formed by a central globular domain and an N-terminal tail with multiple sites for modification of nucleosomal organization, leading to ATP-dependent chromatin remodeling complexes and post-translation aminoacid modifications on histone tails (histone acetylation, methylation, phosphorylation, sumoylation, ubiquitylation, glycosylation, ADP ribosylation, biotinylation). Histone modifications (HMs) are essential epigenetic features, with fundamental roles in biological processes such as transcription, DNA repair and DNA replication. Histone acetylation is achieved by the action of histone acetyltransferase (HAT), which adds an acetyl group to a lysine residue, resulting in chromatin/transcriptional activation; histone deacetylation is produced by histone deacetylases (HDACs) which remove the acetyl groups, and is related to chromatin inactivation and transcriptional repression. Histone acetylation is catalyzed by 5 families of histone lysine acetyltransferases (KATs) (KAT2A/GCN5, KAT2B/PCAF, KAT6-8, CREBBP/CBP, EP300). Histone acetylation is associated with transcriptional activation and open chromatin conformation. In contrast, histone deacetylation is involved in transcriptional repression and closed chromatin structure. In mammals, there are 18 histone deacetylases (HDACs), which are classified into 4 classes according to their homology to yeast. Histone deacetylation is catalyzed by these 4 classes of histone deacetylases (HDACs) (class I, II, III, IV). Class I HDACs (HDAC1, 2, 3, and 8) are nuclear proteins; HDAC1 and HDAC2 are often found in transcriptional corepressor complexes (SIN3A, NuRD, CoREST), and HDAC3 is found in other complexes (SMRT/N-CoR); class II HDACs are subdivided into class IIa (HDAC4, 5,7, and 9), and IIb (HDAC6 and 10), which are located in the nucleus-cytoplasm interface and in the cytoplasm, respectively. Class III HDCAs belong to the sirtuin family, with nuclear (SIRT1, 2, 6, 7), mitocondrial (SIRT3, 4, 5), or cytoplasmic (SIRT1, 2) localization. Class IV HDAC (HDAC11) is a nuclear protein. The structural classification of HDAC inhibitors differentiates several classes: (i) short-chain fatty acids (sodium butyrate, sodium phenyl butyrate, valproic acid, pivaloyloxymethyl butyrate (AN-9, Pivanex))(selective inhibitors of class I HDACs); (ii) hydroxamic acids (suberoylanilide hydroxamic acid (SAHA, Vorinostat), oxamflatin, pyroxamide, trichostatin A (TSA), m-carboxycinnamic acid bis-hydroxamide (CBHA), derivatives of the marine sponge

***Corresponding author:** Ramón Cacabelos, Chair of Genomic Medicine, Camilo José Cela University, Madrid, EuroEspes Biomedical Research Center, Corunna, Spain, E-mail: rcacabelos@ucjc.edu

Received March 19, 2014; **Accepted** March 21, 2014; **Published** March 28, 2014

Citation: Cacabelos R (2014) Metabolomics of Epigenetic Drugs: Precautionary Measures. Metabolomics 4: e126. doi:10.4172/2153-0769.1000e126

Psammaplysilla purpurea (NVP-LAQ824, NVP-LBH589), LBH-589 (Panobinostat), ITF2357 (Givinostat), PXD101 (Belinostat), CHR-3996, CHR-2845, PCI-24781)(inhibitors of class I and II HDACs); (iii) cyclic peptides (depsipeptide FR901228, romidepsin; apicidin, cyclic hydroxamic acid-containing peptides (CHAPS), cyclic tetrapetides trapoxin A and B with the epoxyketone-containing amino acid (2S,9S)-2-amino-8-0xo-9,10-epoxy-decanoyl (Aoe), chlamydocin, HC toxin, bacterial FK228)(class I HDAC inhibitors); (iv) benzamides (MS-275 (Entinostat), CI-994, RGFP136, MGCD0103 (Mocetinostat)) (class I HDAC inhibitors; selective HDAC1 and HDAC3 inhibitors); (v) ketones (trifluoromethyl ketone); (vi) sirtuin inhibitors (Class III HDAC inhibitors) (nicotinamide/niacinamide, suramin); and (vii) miscellaneous compounds (MGCD-0103, natural bioproducts). Several issues limit the clinical use of pan-HDAC inhibitors. They induce apoptotic cell death and cell cycle arrest in tumorigenic and neuronal cells, alter immune function, and may pose additional problems to cross the blood-brain barrier due to their poor penetrance.

Long non-coding (lnc) RNAs are defined as non-protein-coding RNAs, distinct from housekeeping RNAs (tRNAs, rRNAs, and snRNAs) and independent from small RNAs with specific molecular processing machinery (micro- or piwi-RNAs). Over 95% of the eukaryotic genome is transcribed into non-coding RNAs (ncRNAs) and less than 5% is translated. LncRNA-mediated epigenetic regulation depends mainly on lcnRNA interactions with proteins or genomic DNA via RNA secondary structures, and some lncRNAs rely on Watson-Crick base pairing for functional activity. ncRNAs are classified by size into 2 categories: (i) small RNAs (<200 nucleotides): (a) structural RNAs: ribosomal (rRNA), transfer (tRNA), small nuclear RNAs (snRNA); (b) regulatory RNAs: microRNAs (miRNA), small interfering RNAs (siRNA), small nuclear RNAs (snRNA), piwi-interacting RNAs (piRNA), splice junction-associated RNAs; and (ii) long RNAs (lncRNAs) (>200 nucleotides), present in >8000 loci in the human genome: large intergenic non-coding RNAs (lincRNA), natural antisense transcripts (NATs), non-coding RNA expansion repeats, promoter-associated RNAs (PARs), enhancer RNAs (eRNAs), small activating RNAs (saRNAs, RNAa). Small ncRNAs (miRNAs, siRNAs, piRNAs) show mature forms of 20-30 nucleotides (nt) that associate with members of the Argonaute (AGO) superfamily of proteins, the central effectors of RNA interference (RNAi) pathways. miRNAs and siRNAs are posttranscriptional gene silencers, guiding AGO complexes to complementary mRNAs in the cytoplasm, inducing transcript degradation and blocking translation. RNA interference (RNAi) technology may potentially be able to inhibit the protein expression of specific genes by activating a sequence-specific RNA degradation process. Short interfering nucleic acid (siNA), siRNA, dsRNA, miRNA and short hairpin RNA (shRNA) are capable of mediating RNA interference (RNAi) against gene expression. RNAi-based treatments represent a promising therapeutic strategy for many complex disorders. Major problems with RNAi strategies are delivery systems and off-target effects.

The genes involved in the pharmacogenomics of complex disorders potentially treated with epigenetic drugs fall into five major categories: (i) pathogenic genes; (ii) mechanistic genes (associated with the mechanism of action of drugs); (iii) metabolic genes (associated with drug metabolism (phase I (*CYPs*) and phase II reactions (*UGTs*, *NATs*); (iv) transporter genes (associated with drug transporters (*ABCs*, *SLCs*)); and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions. Furthermore, epigenetic regulation is responsible for the tissue-specific expression of genes involved in pharmacogenetic processes, and epigenetics plays a key role in the development of drug resistance. In this regard, to optimize therapeutics with this category of drugs, it is important to understand the reciprocal effects that epigenetic drugs exert on pathogenic, mechanistic, metabolic, and transporter genes. Although this is an still poorly explored field, epigenetic regulation of genes encoding drug-metabolizing enzymes (*CYP1A1*, *1A2*, *1B1*, *1A6*, *2A13*, *2B6*, *2C8*, *2C9*, *2C18*, *2C19*, *2D6*, *2E1*, *2J2*, *2F1*, *2R1*, *2S1*, *2W1*, *3A4*, *3A5*, *3A7*, *3A43*, *UGT1*, *GSTP1*), drug transporters (*ABCB1/MDR1/P-gp*, *ABCC1/MRP1*, *ABCC11/MRP8*, *ABCG2/BCRP*, *SLC19A1*, *SLC22A8*), and nuclear receptors (*RARB2*, *ESR1*, *NR1I2*, *HNF41*) has been documented in pioneering studies of pharmacoepigenetics. All these extremely complex metabolomics networks suggest that pharmacogenetic and pharmacoepigenetic studies are required for the proper evaluation of efficacy and safety issues in clinical trials with epigenetic drugs.

This brief summary of epigenetic therapeutics illustrates the predictable metabolomic outcome when using this novel category of drugs to reverse specific pathogenic cascades. At the present stage of development, epigenetic drugs represent a promising therapeutic aid for the treatment of complex diseases, such as cancer or brain disorders, with many questions to be answered prior to embarking the pharmaceutical industry and the scientific community on another journey of frustrating expectations.