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The impact of epigenomics on future drug design and new therapies

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The future of drug design and the development of new therapeutics will rely on our ability to unravel the complexities of the epigenome in normal and disease states. Proper epigenetic regulation is essential for normal differentiation in embryogenesis and development. Conversely, abnormal epigenetic regulation is a feature of complex diseases, including cancer, diabetes, heart disease and other pathologies. Epigenetic therapies hold promise for a wide range of biological applications, from cancer treatment to the establishment of induced pluripotent stem cells. The creation of more specific and effective epigenetic therapies, however, requires a more complete understanding of epigenomic landscapes. Here, we give a historical overview of the epigenomics field and how epigenetic modifications can affect embryo development and disease etiology. We also discuss the impact of current and future epigenetic drugs.

Introduction

Epigenetics is defined as the study of heritable changes in phenotype that do not involve alterations in the DNA sequence [1–4]. Epigenetic modifications have been documented for over 40 years and they can significantly alter the cellular phenotype due to their ability to mediate gene silencing or gene activation [5,6]. Epigenetic regulation of gene expression is mediated through chromatin: a complex of DNA, histones, non-histone proteins and non-coding RNAs (ncRNAs) that form the structural matrix of a chromosome [7].

There are at least three types of epigenetic modifiers: DNA methylation, histone modifications and ncRNAs. The first type of modification, DNA methylation, directly affects the genomic DNA. It involves the covalent addition of a methyl group to the carbon atom 5 of the cytosine pyrimidine ring in a CpG (cytosine-guanine) dinucleotide [2,8]. However, non-CpG cytosines can have this modification early in development [9]. Methyl groups in the dinucleotide CpGs of gene promoters can turn genes off or on by affecting interactions between DNA and the transcription machinery of the cell.

The second type of modification, post-translational histone modification, indirectly affects DNA. Histone proteins assemble into a protein complex that associates with DNA and forms a structure known as the nucleosome. Histone proteins make up the core of the nucleosome, and DNA coils around this histone core to become more compact and to form higher order chromatin structures. Histone proteins are subject to a myriad of post-translational modifications, including acetylation, ADP ribosylation, citrullination, clipping, methylation, phosphorylation, sumoylation, ubiquitination and others [9–12]. Post-translational modification of histones can alter the electrostatic interaction between the histones and nearby DNA. These changes can alter accessibility to the transcriptional machinery [10]. Histone modifications can also alter the association of histones with protein complexes. For example, bromodomain proteins can bind to acetylated histones; these bromodomain proteins can play a role as a subunit of a protein complex that further modulates chromatin structure and gene transcription [11,12].

The third modification involves different classes of ncRNAs, which can physically bind to the DNA, alter its conformation and, in the case of microRNAs, silence genes by post-translational control [13]. For example, long ncRNAs combined with complexes of proteins, such as polycomb, can bind to the DNA, change the structure of the chromatin and affect gene expression [14]. Furthermore, other types of ncRNA, such as microRNAs and a variety of small RNAs, are able to regulate gene expression through alternate mechanisms [15].

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TABLE 1

Epigenetic and epigenomic associations with complex disease

Diseases and disorders	Relationship between epigenetic/ epigenomic alterations and diseases	Current and future areas of therapeutic intervention	Refs
Alzheimer's disease[a]	Changes in DNA methylation and histone modifications were reported for Alzheimer's disease. For a summary of alterations, see [103]	The association between epigenetic abnormalities and Alzheimer's disease suggests that drugs targeting epigenetic pathways have therapeutic value Animal model studies indicate that HDAC inhibitors might be useful for the treatment of Alzheimer's disease	[103]
Asthma and COPD[a]	Chromatin remodeling affects the expression of inflammation-related genes in COPD and asthma HDAC2 mediates the repression of inflammatory genes; HDAC2 levels are reduced in alveolar macrophages of patients with COPD and asthma	Theophylline can restore HDAC2 activity in COPD macrophages HDAC inhibitors could be used to decrease inflammatory gene expression in COPD and asthma	[67,104,105]
Atherosclerosis	Hypermethylation of specific genes was widely reported; however, hypomethylation has also been detected in advanced human atherosclerotic plaques	Drugs targeting epigenetic changes could provide new avenues of treatment for cardiovascular disease	[106]
Cancer (several types)[a]	See references for a general overview of this complex disease	HDAC inhibitors, DNA methyltransferase inhibitors, small molecules targeting chromatin-associated proteins, and others	[49,102,107]
Coronary artery disease[a]	Global DNA methylation levels significantly higher in patients with coronary artery disease compared with normal controls	ND[b]	[108]
Depression	Early-life stress in rodents can lead to DNA hypomethylation and expression of arginine vasopressin (AVP), which is associated with features of depression Levels of DNA methyltransferase-2 are elevated in a rodent model of depression	Behavioral phenotypes induced by the early-life stress could be reversed by treatment with a AVP V1b receptor antagonist (SSR149415) Imipramine (antidepressant) reversed the downregulation of brain-derived neurotrophic factors and increased histone acetylation at the promoters of these genes	[71,109,110]
	Brain-derived neurotrophic factors III and IV are downregulated in a rodent model of depression, and this downregulation is correlated with increased repressive histone methylation at their corresponding promoters		
Diabetes mellitus (Type 1)[a]	Association of type 1 diabetes mellitus and the imprinted DLK1-MEG3 locus on chromosome 14q32 DNA methylation affects the expression of candidate type 1 diabetes causal genes: insulin precursor (INS), interleukin 2 and 10	ND	[111,112]
Diabetes mellitus (Type 2)[a]	The following epigenetic changes have been documented during the development of type 2 diabetes mellitus in rats: Pdx-1 DNA methylation and histone deacetylation; Glut4 histone deacetylation	Therapies targeting epigenetic gene regulation could be used to treat type 2 diabetes mellitus	[74,75,113]
		Glucagon-like peptide 1, glucose-dependent insulinotropic-peptide 1, and pioglitazone (PPAR-γ agonist) are used to treat patients with type 2 diabetes mellitus and they have been shown to reverse epigenetic modifications *in vitro*	
Endometriosis and implantation failure (pregnancy)	The *HOXA10* gene is hypermethylated in the endometrium of women with endometriosis compared with controls. Silencing of *HOXA10*, partially due to hypermethylation, could lead to impaired embryo implantation in endometriosis	The reversion of aberrant methylation through pharmacological means could be a novel form of treatment for endometriosis and implantation failure	[57,114,115]

TABLE 1 (*Continued*)

Diseases and disorders	Relationship between epigenetic/epigenomic alterations and diseases	Current and future areas of therapeutic intervention	Refs
	DNA methyltransferase 3A is upregulated in the eutopic endometrium of women with endometriosis		
Inherited glycosylphosphatidylinositol (GPI) deficiency	Mutation (C–G substitution) in the GPI mannosyltransferase 1 gene (*PIGM*) results in inherited GPI deficiency. The mutation causes hypoacetylation of the PIGM promoter	The HDAC inhibitor, sodium butyrate, increased *PIGM* expression and alleviated disease-associated symptoms of GPI deficiency in a patient	[116]
Schizophrenia and bipolar disorder	Several loci are epigenetically different in the brains of patients with either schizophrenia or bipolar disorder compared with unaffected controls SNPs in the *HDAC3* and *HDAC4* genes are associated with schizophrenia and could be involved in the pathophysiology of the disease	ND	[117,118]
Additional psychiatric disorders	See [119] for a detailed description of epigenetic and/or epigenomic alterations in Fragile X syndrome, Coffin–Lowry syndrome, Rett syndrome, myotonic dystrophy, Prader–Willi syndrome and Angelman syndrome		[119]

[a] Diseases that are classified as the top ten worldwide causes of death according to the World Health Organization [56].
[b] ND: topic not discussed in the report referenced.

Altogether, several modifications can affect the epigenetic status of a particular locus in the genome. Epigenetic modifications are essential to normal mammalian development and they continue to have a role in gene regulation and genome stability throughout the lifespan of an organism [2]. Given the prevalence and importance of epigenetic alterations throughout development, it is not surprising that epigenetic abnormities contribute to the pathogenesis of human cancers. Abnormal epigenetic alterations were first described in colon cancer [16], but subsequent research in the field of oncology indicated that epigenetic alterations might be one of the most common abnormities in human cancers [2]. Along similar lines, epigenetic abnormalities have been detected in several other human diseases and disorders (Table 1).

The occurrence of epigenetic abnormities in human pathologies indicates that specific diseases might benefit from epigenetic-targeted therapies. In fact, drug therapy aimed at targeting epigenetic defects is becoming a reality in clinical settings [17,18]. The development of research tools to characterize and functionally analyze the variety of epigenetic modifications throughout the entire genome (epigenomics) will provide insight into the function of epigenetic modifications in normal development and in the subsequent transition to disease states, ultimately leading to the future development of more effective epigenetic-based therapies. The purpose of this review article is to give a historical perspective and overview of the emerging field of epigenomics in normal development and disease. This review will also explore the impact that epigenomics will have in drug development for a variety of complex diseases.

A historical perspective: epigenetics and the emergence of epigenomics

Conrad H. Waddington first introduced the term 'epigenetics' during the mid-20th century to describe the variety of developmental phenomena above the level of genomes that connected genotype to phenotype [19]. The word 'epigenetics' was originally derived from the Greek word 'epigenesis', which during the early 20th century was used to describe the changes in morphogenesis and development of organisms [20]. Later on, the term 'epigenesis' was reevaluated and changed to epigenetics [21]. The early definition of epigenetics was entirely based on observations at the level of organism development and was mainly supported by the interactions with the environment during development. The interactions between the genotype and the environment represented what is currently known as the epigenotype [22]. Newman and Muller defined epigenetics as the interactions of cells with each other and with the surrounding microenvironment [23]. Oster and Albert also proposed that physical interactions of tissues and their extracellular matrices are crucial for the developmental process and epigenetic components might have an important role in this process [24]. As recently discussed by Meissner, a wider and contemporary definition of epigenetics would include stable but reversible molecular mechanisms that lead to a given phenotype without changes in the genotype [25]. Currently, it is clear that Waddington's observation at the level of the organism was a consequence of a series of molecular changes that occur in the DNA of the cells after interactions with the microenvironment. A summary of the theories, hypothesis and main discoveries in the epigenetics and epigenomics are shown in Fig. 1.

Several decades after Waddington first introduced a broad definition of the term 'epigenetics', it was suggested that 5-methyl cytosine had a role in regulating gene expression at the molecular level and that the patterns of DNA methylation are somehow heritable [26]. Evidence also indicated that DNA methylation is associated with gene silencing [26]. Interestingly, in 1969 when studying brain memory, Griffith and Mahler made the first suggestion that the gain or loss of DNA methylation has an important biological role [27]. However, in 1975, Holiday and Pugh proposed a molecular model for turning genes on and off based on changes

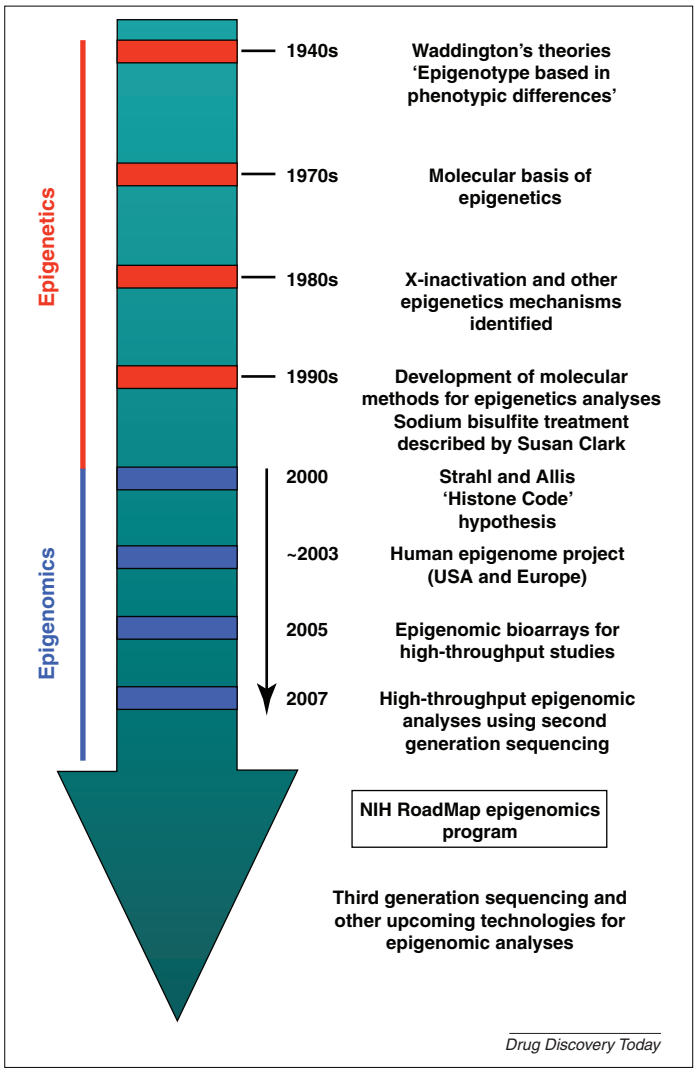

FIGURE 1

Historical perspective of epigenetics and the emergence of epigenomics. The green arrow represents the historical evolution of important breakthroughs in both epigenetics and epigenomics fields. The vertical red line represents the field of epigenetics and the vertical blue line depicts the emergence of epigenomics with the advent of new technologies. Waddington first coined the term 'epigenetics' during the early 1940s and it was used until the end of the 20th century. At the beginning of the 21st century, whole-genome analyses of epigenetic changes (epigenomics) began to be widely used by different groups. This new field of research is still evolving, especially with the launch of high-throughput methods for analyzing epigenomic changes. For example, the National Institutes of Health (NIH) RoadMap Epigenomics Mapping Consortium, which started in 2008, is conducting in-depth epigenomic mapping of several high-priority cell types (for more details, see [120]).

in DNA methylation [28]. Finally, in 1987, Holliday revisited Waddington's ideas and published a crucial article connecting the molecular and phenotypic aspects of epigenetics [29]. It is now clear that epigenetic changes, as pointed out by Waddington several years ago, are important for proper embryo development and mistakes in this program can lead to the transition from normal to pathological states (Fig. 2).

Epigenomics has emerged with the advent of new technologies that enable the analyses of epigenetic modifications of entire genomes. This field was defined by Callinam and Feinberg as a new discipline that studies epigenetic modifications at the molecular level in an entire genome instead of single gene or a smaller number of genes [30]. Epigenomic promises to provide novel insights into genomes because of its potential to detect quantitative alterations, multiplex modifications and regulatory sequences that contribute to gene expression control. Unraveling the intricacies of the epigenome will be a complex process due to the enormity and dynamic nature of the epigenomic landscape. Loci throughout the entire genome might contain multiple epigenetic modifications, and it will be important to determine the cause and effect of these modifications at both the intracellular and extracellular levels.

Novel approaches, such as the development of new DNA sequencing technologies, are facilitating the analyses and understanding of entire epigenomes (see Fig. 1 for details). These technologies can be used to interrogate entire genomes for methylation changes or histone modifications by combining traditional epigenetic analyses with next-generation DNA sequencing. Examples include whole-genome histone modification analyses in normal cells, such as embryonic stem cells [31] and differentiated cells [32]. Additionally, epigenomic approaches are being used to study the methylation landscape of complex diseases, such as cancer [33], psychiatric disorders [34] and other diseases, as discussed below.

Development, stem cells, and iPSCs

During mammalian embryogenesis, the entire maternal genome is altered through passive DNA demethylation and the paternal genome is subject to active DNA demethylation [35] (see also Fig. 2). Most of the mammalian genome is demethylated by the time the morula stage embryo has developed; however, *de novo* DNA methylation is detected in the blastocyst stage embryo [36]. *De novo* epigenetic changes are likely to have a role in controlling cell fate as 'lineage-committed' cells have an increase in repressive chromatin structure with respect to genes that affect pluripotency [37]. Accordingly, normal epigenetic regulation is essential for the appropriate differentiation of embryonic stem cells [38,39].

Epigenetics and induced pluripotent stem cells

Hawkins *et al*. suggest that repressive epigenetic domains represent a key feature of differentiated cells, which act by decreasing the plasticity of the cells [37]. This information is particularly relevant given the recent interest in induced pluripotent stem cell (iPSC) research [40]. iPSCs are differentiated somatic cells that are experimentally reprogrammed to resemble embryonic stem cells [41]. In theory, iPSCs can give rise to cells from all developmental lineages. These cells provide an intriguing system to study normal development as well as disease development. They might also provide regenerative therapies that will be useful for the treatment of many different human diseases [42], but more work is needed to comprehend fully the molecular characteristics of iPSC formation and differentiation. For example, failure to reverse repressive epigenetic domains might limit the potential of somatic cells to be reprogrammed to iPSCs [37]. Indeed, iPSCs retain an aspect of epigenetic memory from their cells of origin, which can affect their capacity to differentiate [43]. However, it should also be noted that iPSCs contain epigenetic marks that are similar to those observed in cancer cells [44,45]. These results make evident that iPSCs have characteristics, mainly epigenetic modifications, which need to be evaluated cautiously during the development of iPSC-based therapeutics.

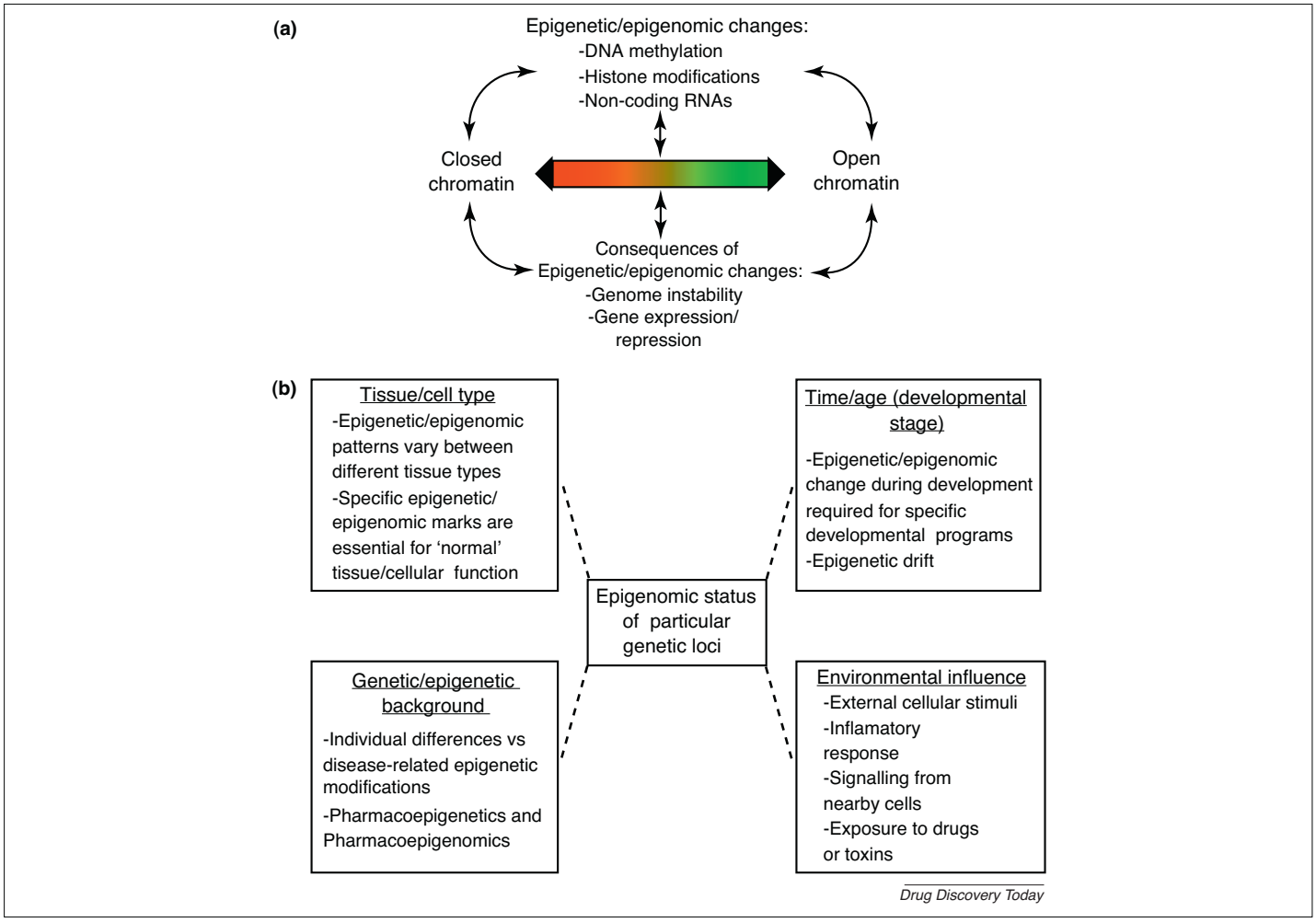

FIGURE 2

Molecular characterization of the epigenetic/epigenomic landscape of cells and the implications for future drug design and new therapies. **(a)** DNA methylation, histone modifications and non-coding RNAs can affect the epigenetic/epigenomic program of cells by promoting a more open or closed chromatin state. Depending on the context, these modifications can lead to activation or repression of both protein-coding and non-coding RNAs (miRNAs, LINE, SINE, long non-coding RNAs and others). Protein-coding RNAs can be translated into proteins that directly affect the epigenome. However, non-coding RNAs can participate in epigenomic signaling through direct and indirect interaction with the chromatin. **(b)** Effective epigenomic drug design necessitates the generation of a complete epigenetic/epigenomic profile for both normal and disease samples. Under normal conditions, the epigenetic and epigenomic patterns might vary between cell/tissue type and also between the same cell type at different time points during development. To design drugs effectively and to predict drug response, it will also be important to identify disease-relevant changes to the epigenome and distinguish them from individual epigenomic variations (this new field of study has been coined 'pharmacoepigenomics'). Finally, it will be important to determine the effect that environmental changes have on the epigenomic signaling. A greater understanding of extracellular cues and their effect on the epigenome will provide insight into the pathways involved in epigenomic signaling and could lead to the discovery of putative drug targets and new therapies. Note: epigenetic drift results from the failure of a cell to transmit or maintain epigenetic modifications and is thought to be associated with aging [51–53].

Taken together, these findings highlight the complex nature of the epigenetic changes that occur during embryonic development, but they are also noteworthy given the increasing interest in iPSC research. Currently, transcription factor transduction is used to create iPSCs, but this process occurs with a very low efficacy [46]. Epigenetic modifying agents induce pluripotency-associated genes and improve the efficiency of iPSC reprogramming [47,48]. Epigenetic modifying agents have been designed for use as chemotherapeutic drugs in diseases, such as cancer [49], but these same drugs could also be used to revert somatic cells to an epigenetic state that more closely resembles that of embryonic stem cells. Existing epigenetic therapies are aimed at bringing on hypomethylation with the goal of reverting hypermethylation-induced gene silencing [50] and, although this would also be a goal of using this type of treatment for iPSC reprogramming, the successful reprogramming of iPSC might require more extensive global changes in the epigenome. Therefore, experiments must be carefully designed when using chemotherapeutic drugs for epigenetic reprogramming, as the goal of this epigenetic treatment is different from that in cancer treatment. As a result, this treatment might require alternate dosages, treatment schedules and combinational approaches.

Epigenetic drift

Studies of monozygotic twins demonstrated that individual epigenomes diverge over time [51]. Over the passage of time, cells

might not fully transmit epigenetic information through cell division; alternatively, a differentiated cell might not have the capacity to maintain its epigenetic profile for an indefinite amount of time [51]. The accumulation of these epigenetic changes with respect to the original epigenome is known as 'epigenetic drift' [51–53]. Epigenetic drift could have significant functional consequences as the 'drifting' of the epigenome might lead to changes in genome integrity or gene expression, which would affect cellular processes, such as aging and disease (Fig. 2). In fact, the findings of Wang *et al.* suggest that epigenetic drift impacts the course of late-onset Alzheimer's disease [54]. The overall effect of epigenetic drift on cellular phenotype is not known; however, this phenomenon should be considered for etiology studies of complex diseases.

Complex diseases and current therapeutics

The ability to dissect the epigenomic landscape is not only essential for a more complete understanding of normal development, but is also necessary to gain insight into the etiology of complex diseases. It is probable that multiple genetic and epigenetic factors contribute to complex diseases (Fig. 2). Adding to this already complicated picture is the likelihood that epigenomic profiles might change throughout the course of the disease. Current epigenetic drugs globally target one specific type of epigenetic abnormality, but complex diseases might arise from a myriad of diverse epigenomic alterations. Globally acting epigenetic drugs might have therapeutic benefit, but a greater understating of the epigenome could lead to more specific and effective therapeutic strategies.

Cancer cells are thought to contain localized regions of DNA hypermethylation (example: tumor suppressor genes) that occurs within a general background of DNA hypomethylation [55]. As a result, the first epigenetic therapies were designed to revert epigenetic abnormities in cancer cells; however, there is growing evidence that epigenetic changes are involved in normal development as well as in a variety of complex human diseases and disorders (Table 1). In fact, several of the leading causes of death, as listed by the World Health Organization [56], are diseases that might have an epigenetic and epigenomic component. Table 1 highlights research that demonstrates epigenetic/epigenomic alterations in a range of diseases. For example, epigenetic abnormities are documented in coronary artery disease (also referred to as cardiovascular disease), diabetes mellitus (type I and type II) and chronic obstructive pulmonary disease (COPD), all of which are significant causes of death throughout the world. Epigenetic/epigenomic alterations are also associated with asthma, Schizophrenia, bipolar disorder, depression, addiction and several other disorders (Table 1). Epigenetic abnormalities might also have a role in infertility, as the impairment of embryo implantation in endometriosis might result from abnormal DNA hypermethylation and the subsequent silencing of HOX genes [57].

Traditional epigenetic therapies

Given the prevalence of epigenetic abnormalities in different types of disease, epigenetic therapy could hold great promise for treatment. Two main classes of current epigenetic therapies are DNA methyltransferase inhibitors and histone deacetylase (HDAC) inhibitors. DNA methylation inhibitors and HDAC inhibitors act globally by promoting a more-open chromatin structure and subsequently they promote gene expression. Global epigenetic therapies are successful in the treatment of myelodysplastic syndrome and cutaneous T-cell lymphoma [58]. These drugs are also useful in combinational therapies, whereby they are thought to enhance the activity of other chemotherapeutic agents [59]. Although global epigenetic therapies have clinical benefits, they nonspecifically alter the epigenomic landscape and might have adverse effects that limit their therapeutic potential.

One potential adverse effect, the activation of genes that are normally epigenetically silenced, could be brought about by DNA hypomethylation and negatively affect disease outcome. Indeed, DNA hypomethylation can promote tumor formation [60,61], and treatment of tumor cells with a global DNA hypomethylating agent, 5′-aza-2′-deoxycytine, can promote aspects of tumor progression [62]. We were able to demonstrate that 5′-aza-2′-deoxycytine promotes global hypomethylation, together with gene-specific DNA hypomethylation [62]. These changes to the epigenome were accompanied by increased invasion *in vitro* and increased tumor growth *in vivo* (Fig. 3) [62]. One particularly important gene, *Sox2*, was not expressed in control cells but became significantly expressed following treatment with this drug. Subsequent analyses revealed that the DNA surrounding the *Sox2* transcriptional start site (TSS) was heavily methylated in control cells but lost this methylation following exposure to 5′-aza-2′-deoxycytine [62]. Given that *Sox2* has a role in pluripotency and iPSC reprogramming [63–65], it is possible that 5′-aza-2′-deoxycytine-mediated *Sox2* expression can lead to the activation of pathways, such as self-renewal, that might negatively affect disease outcome. Taken together, global DNA methylation inhibitors and HDAC inhibitors appear to have clinical benefits in diseases that arise from repressive chromatin-mediated gene silencing. However, treatment with these classes of drugs should be carefully examined to determine whether the therapeutic benefits outweigh the potential adverse effects.

Epigenetic therapy and COPD

DNA methylation inhibitors and HDAC inhibitors might be useful for reverting abnormal gene silencing in a specific disease, but the same drugs might not be useful in the treatment of diseases that result from epigenomic changes that promote gene activation rather than gene repression. For example, patients with COPD have increased levels of histone acetylation in the histones nearby the promoter regions of genes involved in the inflammatory response. In COPD, this increase in acetylation is correlated with inflammatory gene expression and increased severity of disease [66,67]. Corticosteroids are used for the treatment of COPD and they are thought to act, in part, through an epigenetic mechanism: HDAC2 recruitment to the promoter of active inflammatory genes [68]. Patients with COPD can become resistant to corticosteroid treatment, and this is thought to occur through decreased HDAC2 activity. Such activity can be restored by treatment with theophylline [69], which can be mediated by the inhibition of phosphatidylinositol 3-kinase (PI3K)-δ [67,70]. This result indicates that PI3K-δ inhibitors might also restore HDAC2 activity [67] and that epigenetic changes can be induced without the use of drugs that nonspecifically alter the epigenome.

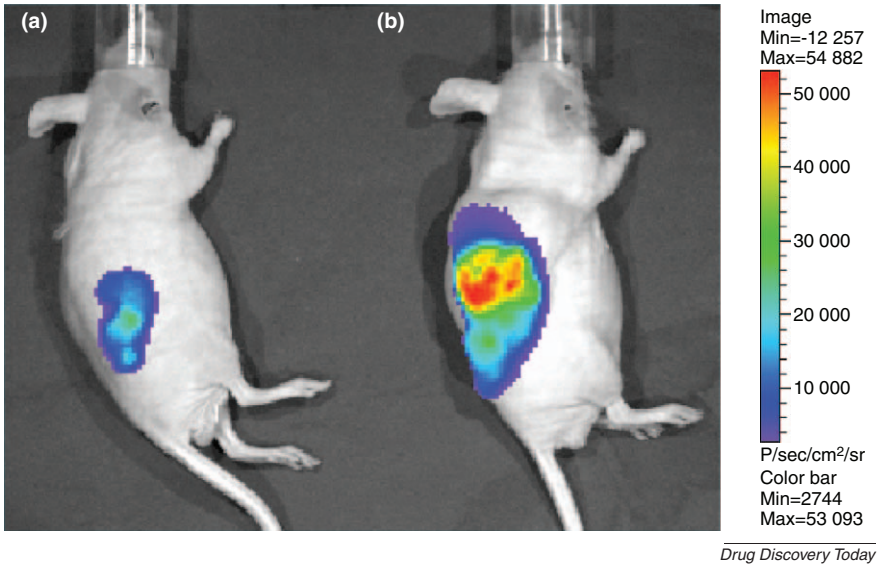

FIGURE 3

Potential concern with drugs that globally alter the epigenome: 5′-aza-2′-deoxycytidine treatment can induce tumor growth and invasion. *In vivo* bioluminescent imaging of tumors in nude mice reveals that treatment with this drug can lead to increased tumor growth. Control cells **(a)** and 5′-aza-2′-deoxycytidine-treated cells **(b)** were injected subcutaneously. The chondrosarcoma cell line was treated *in vitro* with 0.1 μm of 5′-aza-2′-deoxycytidine for 30 days. The drug was removed on the day of the injection and the cells did not receive any further 5′-aza-2′-deoxycytidine treatment. Control cells were grown *in vitro* for 30 days without the drug. This image was collected 6 weeks following tumor induction. Increased tumor growth correlated with increased bioluminescence (displayed using a pseudocolor scale: red, high photon flux; blue, low photon flux). Treatment with 5′-aza-2′-deoxycytidine also increased the invasiveness of the chondrosarcoma cells *in vitro*. This was accompanied by hypomethylation of the *Sox2* promoter and subsequent expression of *Sox2*. For additional information, see [62]. *Note: this is an unpublished image from a previously published study [62] and its purpose is to exemplify the differences in tumor growth following treatment with 5′-aza-2′-deoxycytidine.

COPD provides an example of a disease in which epigenomic alterations have a role in the etiology of the disease and in which treatment involves reverting some of these abnormalities. Initially, corticosteroids and theophylline were used for their clinical benefits but their effect on HDAC2 activity was unknown. Research into mechanisms of action of these drugs revealed their ability to increase HDAC activity and, subsequently, affect epigenetic modifications. Indeed, traditional therapies for other diseases, such as depression and diabetes mellitus, are now shown to act through an epigenetic mechanism.

Epigenetic therapy for depression

The antidepressant, imipramine, can induce the acetylation of histones and reverse depression-induced repressive chromatin modifications [71]. The effect of imipramine appears to be mediated through its ability to inhibit HDAC5 in the hippocampus of a rodent model [71]. It is also noteworthy that, in a mouse model for antidepressant efficacy, the HDAC inhibitor sodium butyrate improved the effectiveness of the antidepressant fluoxetine (a selective serotonin reuptake inhibitor) [72,73].

Epigenetic therapy and diabetes mellitus

The diabetes mellitus drugs glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic-peptide 1 (GIP) can induce global changes in histone acetylation [74,75]. The mechanism of GLP-1 and GIP action appears to be mediated through the ability of these compounds to increase histone H3 acetyltransferase activity and decrease HDAC activity [74,75]. Interestingly, a review by Christensen *et al.* indicates that inappropriate chromatin remodeling and histone acetylation might contribute to diabetes mellitus pathogenesis, and that HDAC inhibitors might have therapeutic potential for diabetes treatment [76].

Taken together, these examples highlight the need to revisit traditional treatments to determine whether a therapeutic benefit occurs through an epigenetic mechanism. As recently discussed by Mohammad and Baylin [77], there is much to learn about the impact that cellular signaling has on the epigenome during development, differentiation and disease. If a drug is found to act through an epigenetic signaling pathway, it could lead to a greater understanding of the disease as well as new avenues for drug design and for more-specific therapies.

New avenues for epigenomic therapy

Inhibition of PAD4

Given the prevalence of epigenetic abnormalities in complex diseases, a greater understanding of the cause and consequence of these abnormalities will translate into benefits in the clinic. Although traditional epigenetic therapies might prove to be beneficial to a range of these diseases, new epigenetic therapies might provide more effective drugs with fewer adverse effects. For example, a newly developed epigenetic therapy targets the protein arginine deiminase 4 (PAD4), a protein that is expressed in a variety of human tumors [78]. PAD4 catalyzes post-translational modification of arginine to citrulline in histone proteins (H2A, H3 and H4), and this modification is associated with transcriptional repression [79]. Inhibition of PAD4 signaling might be useful for cancer treatment, as two PAD4 inhibitors, F-amidine and Cl-amidine, can decrease cell viability and enhance the effectiveness of doxorubicin [80]. In addition, the

discovery of abnormal PAD4 activity in rheumatoid arthritis and multiple sclerosis [81] suggests that therapies targeting PAD4 will also be useful for the treatment of these diseases.

DNA demethylases

The recent discovery of enzymes with DNA demethylating activity has also opened the door to potential areas of epigenomic drug design. Both activation-induced cytidine deaminase (AID) and Tet1 have DNA demethylase activity [82,83]. AID has a role in the active demethylation of primordial germ cells [84], and is required for active DNA demethylation during iPSC reprogramming [82]. DNA demethylases have the potential to affect many aspects of normal development and disease states. Indeed, aberrant AID expression is associated with colon cancer [85]. The role of AID in other diseases is not well documented, but the design of drugs that affect AID activity could be of relevance to diseases with an epigenomic component.

Repetitive elements

Research on PAD4 and AID provided a greater understanding of specific aspects of epigenomics, but more research is needed to reveal the relationship between epigenomics and the development of human diseases. For example, a relatively unstudied phenomenon that might contribute to disease pertains to the ability of cellular stresses and the microenvironment to promote significant changes throughout the epigenome [86]. However, it is unknown to what extent these changes impact disease etiology. For instance, cellular stress can lead to the expression of retrotransposable elements, which is related to their epigenetic regulation [87–89]. The expression of these elements might have a drastic impact at the cellular level, given that repetitive element expression is correlated with genomic instability [90], RNA interference and/or regulation [91–93] and human disease [94]. Based on the abundance and potential function of repetitive elements, given that long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs) comprise at least 34% of human genomic DNA [95,96], it is possible that the abnormal epigenetic regulation of these elements could have a significant impact on the initiation and progression of human disease. Although it might be difficult to prevent epigenetic changes that promote repetitive element expression, it might be possible to design drugs that target the downstream effect of the changes in epigenetic regulation. For example, we hypothesize that polymerase III inhibitors are a suitable alternative to abrogate the epigenetically mediated repetitive element expression. It is also possible to envision therapies that target the specific epigenomic abnormality, or the genes and/or pathways that act upstream or downstream of the aberrant epigenomic modification. Independent of the specific target, the treatment should ameliorate the effects of the abnormal epigenomic modifications.

Overall, to design epigenomic therapies effectively, it will be necessary to obtain a complete molecular profile of the epigenomic landscape in both normal and disease states. Combining traditional epigenomic [DNA methylation and chromatin immunoprecipitation or (ChiP)] analysis with next-generation DNA sequencing will help define the epigenomic status of particular cell types (Fig. 1). It is important to note that epigenomic profiling should truly be global, and focus on regions containing protein-coding sequences as well as other regions of the genome (e.g. regulatory sequences, ncRNAs, and repetitive elements). This type of epigenomic profiling will lead to the identification of disease-associated epigenomic changes and to the identification of new therapeutic targets. For example, epigenetic and gene expression profiles from liver cancer cell lines can be used to predict the therapeutic response of cancer cells to a DNA methyltransferase (DNMT) inhibitor [97]. These data indicate that epigenomic profiling and gene expression profiling might become common approaches for clinical interventions and disease management. In fact, epigenomic profiling is fostering in the era of pharmacoepigenetics and phamacoepigenomics, fields that involve the study of the relationship between the epigenome and optimal drug dosage and/or response, with a goal of optimizing individualized treatment and discovering new drug targets [98,99].

Conclusions and future prospects

The importance of epigenetic regulation is highlighted by, but not limited to, its function in embryogenesis and disease as discussed. Our knowledge of epigenomics is in its infancy, and a greater understanding of the causes and consequences of these changes will lead to the identification of new drug targets and therapies. The continued development of new sequencing technologies will aid in high resolution mapping of the epigenomic landscape. At the same time, it will be important to identify novel signaling pathways that might act as modifiers of the epigenome. For example, large intergenic ncRNAs (lincRNAs) have recently been shown to bind to chromatin-modifying complexes and they have been implicated in p53-mediated gene repression [100,101]. LincRNAs might be an integral part of epigenomic signaling pathways; however, more research is needed to elucidate the function of these RNAs in normal development and disease. ncRNAs are becoming known as masters of gene regulation and their importance in the field of epigenetics is growing fast [102]. Thus, a greater understanding of epigenomics and the factors that mediate changes to the epigenome will lead to a better knowledge of gene regulation and will also translate into more effective disease treatments.

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