

Epigenomics in cancer management

Fabricio F Costa

Cancer Biology and Epigenomics Program, Children's Memorial Research Center and Northwestern University's Feinberg School of Medicine, 2430 N. Halsted St, Box 220, Chicago, IL, USA

Abstract: The identification of all epigenetic modifications implicated in gene expression is the next step for a better understanding of human biology in both normal and pathological states. This field is referred to as epigenomics, and it is defined as epigenetic changes (ie, DNA methylation, histone modifications and regulation by noncoding RNAs such as microRNAs) on a genomic scale rather than a single gene. Epigenetics modulate the structure of the chromatin, thereby affecting the transcription of genes in the genome. Different studies have already identified changes in epigenetic modifications in a few genes in specific pathways in cancers. Based on these epigenetic changes, drugs against different types of tumors were developed, which mainly target epimutations in the genome. Examples include DNA methylation inhibitors, histone modification inhibitors, and small molecules that target chromatin-remodeling proteins. However, these drugs are not specific, and side effects are a major problem; therefore, new DNA sequencing technologies combined with epigenomic tools have the potential to identify novel biomarkers and better molecular targets to treat cancers. The purpose of this review is to discuss current and emerging epigenomic tools and to address how these new technologies may impact the future of cancer management.

Keywords: genomics, epigenomics, epigenetics, DNA methylation, histone modifications, new technologies, cancer management

Introduction

Sequencing the human genome marked not the end of the field of genomics, but its beginning. Scientists now stand in a unique position in the history of medicine to define human disease, armed with the technological advancements and the data that has resulted from the Human Genome Project.^{1,2} Using this information, some progress has already been made toward launching individualized and personalized medicine, especially for certain types of cancer. For example, the launch of different types of genetic tests to predict disease (preventive medicine) and the development of the drug imatinib (Gleevec[®]) for blood tumors³ were major breakthroughs. In addition, biomarkers that are able to subgroup tumors based on aggressivity, thus aiding in clinical decisions, were also identified.⁴ A precise molecular characterization of human cancers will allow a better understanding of the basis for disease susceptibility and environmental influence, better diagnosis and prognosis, and the refinement of individualized treatment for optimal therapeutic efficacy.

Genomes from various individuals have already been sequenced,^{5,6} allowing genomic comparisons. Projects such as the HapMap⁷ that identified variations in the human genome, and ENCODE⁸ that is analyzing the functional elements in the genome, are helping in the understanding of complex disease phenotypes. Such projects have the

Correspondence: Fabricio F Costa
Cancer Biology and Epigenomics Program,
Children's Memorial Research Center
and Northwestern University's Feinberg
School of Medicine, 2430 N. Halsted St,
Box 220, Chicago, IL, USA
Tel +1 773 880 4000 ext. 57312
Fax +1 773 755 6551
Email fcosta@childrensmemorial.org

potential to help elucidate the information encoded by human genomes and aid in the treatment of diseases such as cancer.

One of the main issues in genomic science is understanding how gene expression is regulated. To understand the mechanisms that are implicated in gene regulation, the genes that are expressed in each cell type of the body and how changes in their expression will impact in the development of diseases represent major challenges. In addition, environmental factors such as the exposure to chemical compounds during life, smoking, and nutrition can clearly affect and change the expression of genes.⁹ Thus, in the post-genomic era, studies of how human genes are regulated and the mechanisms that are implicated in this process are of major importance for our understanding of normal processes and diseased states.

The information beyond the genome sequence was recently coined as the epigenome.¹⁰ The epigenome is defined as the group of modifications that can occur at a genomic level that will not change the sequence of the bases of the DNA but can change the DNA conformation and, as a consequence, change the expression of genes. Epigenetics is the study of these modifications in the DNA.¹¹ The following

are the main epigenetic modifications that occur in the DNA molecule: 1) binding of different proteins to the DNA such as histones and methyl-binding proteins, 2) addition of chemical groups in the bases of the DNA such as methyl (CH₃), and 3) microRNAs and other noncoding RNAs that can regulate the expression of genes through various mechanisms.

While epigenetics has garnered more attention, it is not a new field, and studies dating from the 1980s have shown the promise of using drugs that affect these mechanisms to treat diseases, especially cancers.^{12,13} In the last decade, we have been facing an overwhelming increase in drugs affecting epigenetic mechanisms that have been developed to treat different types of cancer (see Table 1 and Figure 1). Examples are a growing number of DNA methylation inhibitors, histone modification inhibitors, and small molecules that target chromatin-remodeling proteins. 5-Azacytidine was the first inhibitor of an enzyme implicated in epigenetic modifications described.¹⁴ This drug inhibits the DNA methyltransferase (DNMT) enzyme that is responsible for adding methyl groups to cytosines located in both DNA and RNA molecules. Another example of a DNMT inhibitor is

Table 1 Drugs developed using epigenetics and epigenomics tools

Drug	Mode of action	Types of cancer	References
Belinostat ¹	Histone deacetylase inhibitor	Hematological malignancies and solid tumors	71,72
Dacogen ^{®1} or Vidaza ^{®2} and decitabine ²	DNA methyltransferase inhibitors (5-azacytidine and 5-aza-2'-deoxycytidine)	Myelodysplastic syndrome and hematological malignancies. Tests have already started in solid tumors	38,73,74
DZNep ¹ (Deazaneplanocin A)	Histone methyltransferase inhibitor	Acute myeloid leukemia	75
Entinostat ¹ (MS-275)	Benzamide histone acetylase inhibitor	Blood and lung tumors	72,76
Panobinostat ¹ (LBH589)	Inhibitor of the enzyme histone deacetylase in a mechanism leading to apoptosis of malignant cells <i>via</i> multiple pathways	Cutaneous T-cell lymphoma, chronic myelogenous leukemia, myelodysplastic syndromes, breast cancer, pancreatic cancer, and prostate cancer	77–79
RG108 ³	Small molecule specifically designed to bind and inhibit the active domain of the DNA methyltransferase I enzyme	Different types of cancer	80
CP-4200 ³	Molecule conjugated to a lipid chain linked to azacytidine that accelerates cellular uptake	Different types of cancer	81
S110 ³	Modified and less toxic version of 5-aza-2'-deoxycytidine; DNA methyltransferase inhibitor	Different types of cancer	82
Romidepsin ¹	Natural product that inhibits histone deacetylases and causes cancer cell apoptosis	Under clinical trials to cutaneous T-cell lymphoma, peripheral T-cell lymphoma, and a variety of other cancers	83
Valproic Acid ² (Depakote [®])	Histone deacetylase inhibitor	Multiple myeloma, gliomas, and melanoma	84
Vorinostat ² (Solinza [®])	Histone deacetylase inhibitor	Lymphomas, glioblastoma multiforme, and other solid tumors	72,85
Pyroxamide ² (SAHA)	Histone deacetylase inhibitor	Hematological malignancies, prostate cancer, bladder cancer, and neuroblastoma	86
Sirtinol and Salermide ³	SIRT1 protein inhibitors	Different types of cancer	43,87

Notes: There might be other examples of epigenetic-based drugs under development and/or in clinical trials that were not described here. ¹In clinical trials; ²Approved by the FDA; ³Under development.

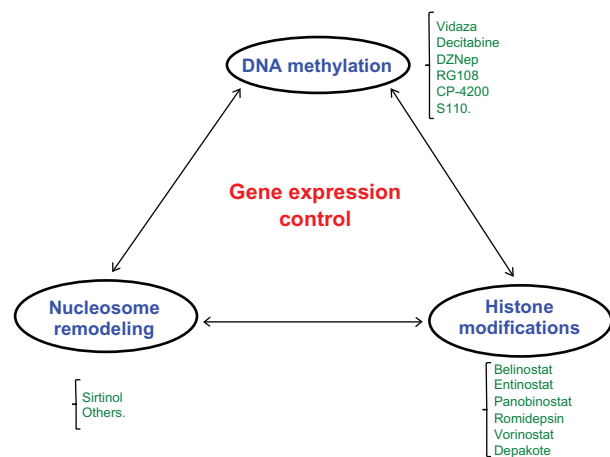


Figure 1 Epigenomics and cancer therapy. Schematic representation of the three main epigenetic/epigenetic components that are implicated in gene expression control in human cells (blue). Some drugs that are in current use, under development, and in clinical trials for each epigenetic mechanism of gene regulation are shown in green. The drugs presented here are just a representative list for each mechanism, and some drugs under development and in clinical trials at the moment are not shown.

5-aza-2'-deoxycytidine, which is incorporated just in the DNA molecule. The DNA methyltransferase covalently binds to these nucleotide analogs, and this sequestration affects its normal function. These compounds can also affect the way proteins implicated in cell regulation are able to bind to the DNA/RNA substrates. 5-Azacytidine was first tested in myelodysplastic syndrome and leukemia, and it showed promising results in patients with both diseases.^{15,16} Since 5-azacytidine and other epigenetic drugs are not very specific, side effects are a major problem. A challenge faced by researchers in this field is two-fold: design more specific drugs and drugs that have fewer side effects since they have a global effect in the epigenome of the cells. To overcome this issue, new DNA sequencing technologies (second and third generation) combined with epigenomic tools have emerged. It is becoming clear that these technologies may facilitate the identification of better molecular targets for drug development and biomarker identification for cancer management. In that regard, the main purpose of this review is to discuss the emergence of epigenomic tools derived from new DNA sequencing technologies and how they may affect the management of cancer in the future.

Cancer genomics – now and then

Genomics is defined as the study of entire genomes of organisms, including extrachromosomal DNA such as the mitochondrial genetic material. This field includes intensive efforts to determine the entire DNA sequence of organisms, using fine-scale genetic mapping and DNA sequencing with current and emerging technologies. In contrast, investigating

the roles of single genes is a primary focus of genetics. Single gene research does not fall into the definition of genomics unless the aim is to verify the effect that a gene may have on the entire genome's networks and pathways. Genomics has been the main focus in molecular biology, especially after the completion of the sequencing of genomes from several organisms. Genomics tools have already helped in the understanding of several aspects of the genome of cancer cells when compared to normal controls. One important example is the identification of the gene *HER2/neu (ErbB-2)*, which is an oncogene mapped to human chromosome 17 that is over-expressed, or amplified, in ~30% of breast cancer tumors.¹⁷ Identification of this molecular characteristic culminated in the development of the drug trastuzumab (Herceptin®).¹⁸ Breast cancer patients that are *HER2/neu (ErbB-2)* positive have increased survival rates when treated with this drug.¹⁸

One question that has recently emerged after the sequencing of entire human genomes and comparisons between them is 'What have we learned from sequencing human genomes?' It is clear that important advances were generated after the sequencing of the first human genome a decade ago. These advancements were mainly applied to basic science such as improvements in DNA sequencing technologies, a decrease in the cost for sequencing the DNA molecule, and also the development of new methods to analyze molecular changes in diseases; for example, there was an increase in the number of predictive and prognostic genetic tests for cancers and other diseases. In contrast, for applied medicine and patients, there have been a disappointingly small number of drugs and therapies that were discovered and developed using genomics tools. Hence, we have learned that the sequence of human genomes is just a starting point to generate and accumulate the basic information needed for more complex and deeper analyses.

Recently, cancer genomes were sequenced and compared with normal cells for leukemia, breast, lung, and other tumor types, using second-generation DNA sequencing technologies.^{19–25} The purpose was to identify mutations that could give rise to new biomarkers and new therapies for these types of cancers. In addition, the 1000 Genome Project was recently launched²⁶ with the objective of sequencing the genome of thousands of individuals in a small period of time. In parallel, companies are starting to provide whole genome sequencing services, with the aim of understanding the individual's susceptibility for diseases, including different cancers.²⁷ As a result, a large number of individuals will have their genomes sequenced in the years to come. But is this enough to understand how the human cell machinery works

and to determine how cancer or any other disease arises? It is likely that the technology will be beneficial to genomic science in the future by paving new ways of approaching diseases, especially cancers. Even though the genomic information will be of importance to identify mutations and other chromosomal abnormalities (ie, insertions or deletions in the genomic DNA of diseased cells), more studies will be necessary to completely understand human genomes. In this regard, the big challenge will be to identify and catalog all the genes that are present in normal cells and their defects (mutations, deletions, insertions, amplifications, fusion proteins, etc) in tumors. There is growing evidence that epigenomics will contribute to this understanding, and in the following sections, I will discuss drugs based on epigenetic mechanisms that are currently in use, in clinical trials, and under development. Furthermore, I will also discuss how this field could aid in the development of better therapies for cancer and in the identification of new biomarkers.

Epigenomics and new therapies

Epigenomics is becoming more important as the technologies for genome-wide epigenetic modification analyses improve. The best-known epigenetic marker is DNA methylation, and some genes are already described with this epigenetic change in different tumor types.²⁸ DNA methylation occurs in normal cells mainly in regions that are intergenic, and loss of methylation (hypomethylation) in these areas was first reported in the 1980s.²⁹ Loss of DNA methylation or global hypomethylation is an early event in cancer, and when it occurs in repetitive regions of the genome, the consequences can be chromosomal instability, as previously described.³⁰ On the other hand, gain of DNA methylation (hypermethylation) in the promoter region of different genes can lead to a decrease in the expression of this gene (also known as downregulation) and can be one of the causes for cancer development.³¹ It is hypothesized that DNA methylation may affect gene expression by blocking transcription after methyl-binding and other proteins form a complex that blocks the access for transcription factors to the gene promoter.³² However, there are other models suggesting that DNA methylation is the consequence instead of the cause of gene inactivation.³³ In this case, defects in the transcription factors such as mutations will leave promoter regions ‘opened’ and more susceptible to the action of the DNMTs.³³ Consequently, these regions of the DNA will be methylated.³³

Different genes are already described as hypermethylated in the initiation and progression of several types of tumors.³⁴ The enzymes responsible for the control of DNA methylation in eukaryotic cells are the DNMTs.³⁵ There are three

enzymes already described: DNMTs 1, 2, and 3. However, DNMT1 may be the most important, especially in diseases such as cancer.³⁶ Different types of drugs that target DNMTs have already been developed. The idea behind using a drug that blocks the enzymes that control DNA methylation is that in cancer, there is an increase in their activity.³⁷ Drugs that block DNMTs include 5-azacytidine, 5-aza-2'-deoxycytidine, small molecule inhibitors, and others (see Table 1 for more details). 5-Azacytidine (Vidaza®) was approved by the Food and Drug Administration (FDA) since it increased the survival of patients with myelodysplastic syndrome, and many patients on Vidaza became transfusion independent, showing the potential for this type of therapy.³⁸

Histone marks were recently described as an important epigenetic modification to control gene expression in normal cells.³⁹ Histone modifications, such as methylation, acetylation, ADP-ribosylation, ubiquitination, phosphorylation, and others, to histone tails alter chromatin structure. However, a complete understanding of the precise molecular mechanisms by which these alterations to histone tails influence DNA-histone interactions remains elusive. There are two main hypothesis on how histone modifications can affect chromosome function: 1) they may alter the electrostatic charge of the histone, resulting in a structural change in histones or their binding to DNA; or 2) these modifications are binding sites for protein recognition motifs, such as the bromodomains or chromodomains, that recognize acetylated lysines or methylated lysines, respectively.⁴⁰ The existence of these modifications and recognition motifs led to the ‘histone code’ hypothesis proposed by Strahl and Allis.⁴¹ Overall, post-translational modifications of histones create an epigenetic mechanism for the regulation of a variety of normal and disease-related processes, including cancers. Drugs affecting histone modifications have been already developed and showed promising results in the treatment for different tumor types (see Table 1 for more details).

Based on this knowledge, different types of histone deacetylase inhibitors, DNMT inhibitors, and small molecules that block enzymes that are implicated in these epigenetic mechanisms have been developed. In addition, combining conventional therapies to drugs that affect epigenetic mechanisms is becoming common. For example, clinical trials using a combination of DNMT inhibitors with conventional chemotherapy were well tolerated in cancer patients and showed encouraging results when compared with chemotherapy alone.⁴²

Drugs targeting chromatin and nucleosome remodeling proteins are also a promising therapeutic strategy to treat human cancers (Table 1). Some of these proteins

are deregulated in cancer, such as sirtuin 1 (SIRT1). It was recently shown that targeting this protein with drugs such as Salermide or Sirtinol can lead to the reactivation of pro-apoptotic genes that are epigenetically repressed exclusively in cancer cells.⁴³ These drugs are also promising as an anticancer agent, providing molecular evidences that SIRT1 might be involved in human tumorigenesis.⁴³ Chromatin-remodeling proteins are important for proper gene expression, and new drugs targeting these proteins will be developed generating more effective therapies against cancer. All the epigenetic/epigenomic mechanisms and some of the drugs that have been tested for cancer therapies are represented in Figure 1.

The impact of new technologies on cancer research

Second-generation DNA sequencing technologies have been used to identify and detect genetic and genomic changes in tumors when compared with normal cells. In the epigenomics field, these technologies have been helpful in identifying regions of the DNA that are differentially methylated and have different histone marks.⁴⁴ The identification of proteins

that are responsible for wrapping the nucleosome of tumor cells was also possible⁴⁴ (see Table 2). The development of new technologies to study cancer epigenomes will be crucial for the identification of defects in tumor cells.

Thirty years ago, sodium bisulfite was first described as a reagent that could be used to detect DNA methylation in specific regions of the DNA.⁴⁵ This discovery has revolutionized the way we have been analyzing DNA methylation changes in cancer cells from different tumor types.⁴⁵ This technology allowed the analyses of specific regions of the DNA, the so-called gene-by-gene analyses (Table 2), to evaluate the percentages of DNA methylation and correlate it to gene expression. In addition to sodium bisulfite treatment, digestion with methylation-sensitive restriction enzymes and several different methods using restriction enzymes combined with polymerase chain reaction⁴⁶ have been used for years. Some limitations of these methods include the low number of dinucleotide CGs or CpGs that can be analyzed at a time (Table 2). Analyses of individual genes and/or regions of the DNA may also be applied to evaluate histone marks and nucleosome packaging, with the use of antibodies against specific marks in the histone proteins. These procedures, however,

Table 2 Different types of technologies to uncover epigenomic changes in cancer

Method(s)	Description	Examples	References
Gene-by-gene analyses	Different methods that are used to evaluate methylation status of gene promoters. The most utilized technology is based in sodium bisulfite treatment that converts unmethylated cytosines to uracil by deamination. Methylation changes are easily detected using this method after DNA sequencing. Other methods include digestion with MSRE and antibodies against methyl-binding proteins that can be used to detect specific methylation changes.	MSRE digestion, bisulfite sequencing, MSP, MethyLight, and others	45,46,88,89
ChiP-ChiP arrays	Specific antibodies are used for proteins (ie, histones) that are binding to the DNA followed by array hybridization. Mainly used to identify regions that are active and/or inactive based on epigenetic modifications.	Chromatin immunoprecipitation combined with microarray hybridization	90
ChiP-Seq technology	Specific antibodies are used for proteins (ie, histones) that are binding to the DNA followed by DNA sequencing to map the locations of the histone proteins in the genome and their specific modifications. Second-generation DNA sequencing has been used to uncover these changes.	Chromatin immunoprecipitation combined with DNA sequencing	91
DNA methylation arrays	Different types of DNA methylation arrays have been used such as arrays containing CG-rich regions of the DNA. Whole genome arrays are also generated after bisulfite conversion of the DNA.	CpG island specific arrays, whole genome bisulfite arrays	92,93
Second-generation DNA sequencing	Methodologies based on pyrosequencing and other technologies are allowing the generation of huge amounts of genomic and transcriptomic data. They have been also used to detect epigenomic modifications in human genomes.	Pyrosequencing, sequencing by oligo ligation and detection, sequencing by synthesis, and others	94,95
Third-generation DNA sequencing	This new generation comprises methodologies that will be available soon mainly based in nanotechnology (ie, nanopores and nanodetectors). These new methods have the potential to decrease the costs of sequencing a genome in a faster way than the current technologies. Applications for epigenomic analyses have been recently reported.	SMRT, nanosequencing, and others	47,48,96–99

Abbreviations: ChiP, chromatin immunoprecipitation; MSP, methylation-specific PCR; MSRE, methylation-sensitive restriction enzymes; SMRT, single molecule real time PCR.

are very laborious and restricted to the region(s) of interest. Recent methods were developed for epigenomic analyses, which can evaluate epigenetic changes on a global level in the genome of tumor cells (Table 2). Examples include chromatin immunoprecipitation combined with DNA sequencing (ChIP-Seq) and high-throughput DNA methylation analyses after sodium bisulfite treatment using new DNA sequencing technologies (Table 2). Second-generation DNA sequencing methods are mainly based on pyrosequencing and emulsion polymerase chain reaction combined with beads that are embedded in slides with small pores (for more information on second-generation DNA sequencing, see Table 2).

A wave of new sequencing technologies, named third-generation DNA sequencing, have been developed with the promise of sequencing genomes, transcriptomes, and epigenomes faster and with lower costs. Some of these technologies are based on the so-called nanopores (Table 2). These pores are small holes that could be biological or solid, in which the DNA can pass and be detected in a controlled manner.⁴⁷ These technologies rely on the detection of single molecules, and labeling of the sequencing substrate is sometimes required. It is possible that these new sequencers developed using nanotechnology could read long stretches of DNA in a greater or comparable way to the technologies that are currently available. A recent report has demonstrated that it is already possible to detect DNA methylation changes without the use of the reagent sodium bisulfite (which degrades the DNA and usually requires high amounts of starting material).⁴⁸ This is possible in a single molecule real time sequencing reaction with nanodetectors.⁴⁸ It is becoming clear that the new technologies under development will be of importance for research in epigenomics. This will have a positive impact on cancer research, facilitating the identification of new biomarkers and drug targets.

Implications for cancer management

The stratification of patients based on their tumor profile and/or specific biomarkers is becoming the best way to subgroup individuals with the same tumor characteristics. This field is also known as personalized or individualized medicine, and its objective is to associate the best treatment for each specific patient or group of patients. Personalized medicine involves the systematic use of molecular information about each individual patient to select or optimize preventative and therapeutic care. These new approaches are changing the way in which pharmaceutical companies try to identify and test new cancer drugs. The idea of a blockbuster drug that could

treat a broad spectrum of tumor types has become unlikely; cancer is a complex disease, and even a specific cancer type, such as breast cancer, has a variety of subclasses with completely different pathological and molecular features.

Epigenomics is a unique approach to cancer research since it can help in the identification of groups of patients with the same epigenetic changes and characteristics in their tumors. The epigenetic drugs used today are unspecific and have side effects such as the ones that occur in conventional chemotherapy. This can vary from patient to patient, depending on the dosage that is prescribed. Importantly, lower dose treatments can reduce the side effects. The identification of DNA methylation and histone modifications associated with cancer may have important clinical utility in the future. The development of new technologies to uncover these changes in a high-throughput fashion will have a major impact as discussed above and shown in Tables 2 and 3.

Some advancement in the field of epigenetics and epigenomics has already led to the identification of specific biomarkers to manage the disease (see Table 3 for more details). A variety of genes have been described as hypermethylated and/or hypomethylated in cancers, and this feature has shown some clinical significance in specific tumor types.^{49,50} Genes such as *GSTPI*, which is hypermethylated in a high percentage of prostate cancers, has been used as a biomarker for this disease in body fluids and biopsy specimens.⁵¹ In addition, groups of genes from the same pathway and/or network have shown the same epigenetic changes in tumors. Examples include genes implicated in cell adhesion, DNA repair, and apoptosis that can be downregulated by DNA methylation. Downregulation of genes associated with cell adhesion and migration can increase the risk of the tumor cells to metastasize to a secondary site in the body.^{52,53} For example, our group has already shown that the adhesion molecule *ADAM23* is highly methylated in breast tumors, and this feature is correlated to metastases and a poor prognosis in breast tumors.^{54,55} A classic example of a DNA repair gene downregulated by DNA methylation is the *MGMT* gene, which is silenced by epigenetic mechanisms in brain tumors.⁵⁶ Tumors that do not express the gene *MGMT* are more sensitive to radiotherapy and chemotherapy with temozolomide, and this molecular feature has been used in clinical decisions and disease management for glioblastomas.^{56,57} Changes in the profile of histone modifications have also been used to evaluate and manage the risk of prostate cancer recurrence in patients.⁵⁸

Another group of genes in which epigenetic changes can be monitored to manage cancer risk and progression is the

Table 3 Some examples of epigenetic and epigenomic changes in single genes or group of genes and their potential impact in cancer management

Gene(s)	Epigenetic/epigenomic changes	Impact for cancer management	References
<i>GSTP1</i>	Hypermethylated in 80%–90% of prostate cancers and unmethylated in benign hyperplasic tissue.	Detection of hypermethylated <i>GSTP1</i> could help to distinguish normal prostate cells from cancer cells. It could also be used as a biomarker in body fluids and biopsy specimens.	51
<i>PI6ink4A</i>	One of the most common tumor suppressors inactivated by DNA methylation in tumors. Hypermethylation has been linked to poor outcome in different types of cancer.	<i>p16ink4A</i> could be used as prognostic marker in cancer.	100
Apoptosis and cell cycle genes (ie, <i>DAPK</i> , <i>p73</i> , and others)	Hypermethylation has been linked to poor outcome in different types of cancer.	Genes associated to apoptosis that are hypermethylated in cancers could be used as prognostic markers.	101–103
Adhesion molecules (ie, <i>cadherins</i> , <i>ADAM23</i> , <i>ADAM33</i> , and others)	Hypermethylated in different types of cancer and associated with cancer metastasis.	Genes associated to cell adhesion could be used as markers for disease progression.	52–55,104
DNA repair genes (ie, <i>MGMT</i> , <i>hMLH1</i> , <i>BRCA1</i> , and others)	Hypermethylation of genes implicated in DNA repair could help in identifying tumors that are more susceptible to therapies such as radiotherapy helping in personalized treatment.	The use of individualized therapies could aid in patient outcome.	56,57,105
Histones	Differential histone modifications such as acetylation and methylation are associated to cancer recurrence and a worse prognosis.	The identification of patients that are at more risk of recurrence of the disease could help in decisions related to treatment and a better follow-up into the clinic.	58
miRNAs	DNA methylation and histone modifications of miRNA genes has been reported by different groups.	miRNAs are noncoding genes that can regulate several proteins in a cellular network and/or pathway. Reexpression of miRNAs in tumors may have an impact for the regulation of key genes in the cells.	60,70,106

Abbreviations: *ADAM23*, a desintegrin and metalloprotease domain 23; *ADAM33*, a desintegrin and metalloprotease domain 33; *BRCA1*, breast cancer gene 1; *DAPK*, Death-associated protein kinase; *GSTP1*, glutathione S-transferase P1; *hMLH1*, human mutL homolog 1; *MGMT*, O6-methylguanine-DNA methyltransferase; miRNAs, microRNAs.

microRNA (miR) gene family.⁵⁹ Some studies have already shown that miRs can be regulated by epigenetic mechanisms (Table 3). Changes in DNA methylation have been reported in specific cancer types for different miRs.⁶⁰ Additionally, miRs are associated with important embryonic gene pathways in cancer, and this connection between embryonic development and cancer should be carefully examined for drug development in the future.⁶¹ Since miRs regulate hundreds to thousands of protein-coding genes by incomplete base-pairing,^{62–64} allowing them to affect networks and pathways of genes, it will be of importance to monitor miR expression changes mediated by epigenetics during cancer initiation and progression.

In the case of drug development and new therapies, it is likely that the future of epigenetic therapy will include the use of multiple drugs that individually have little effect in epigenetic silencing but that might be expected to have synergistic and/or additional effects when combined. For example, a recent study using histone deacetylase inhibitors and high-dose chemotherapy both in vitro and in vivo indicated that this combination might overcome chemoresistance, achieve durable remission, and improve survival of patients

with Burkitt lymphoma.⁶⁵ A major problem with the use of current epigenetic drugs is that they are nonspecific and can reactivate genes randomly. A concern is that they can cause a whole-genome hypomethylation, increase the number of chromosomal abnormalities, and affect the tumorigenic phenotype of cancer cells as previously described.^{66,67} However, evidence that DNA methylation inhibitors act only in dividing cells, leaving nondividing cells unaffected, has already been reported.⁶⁸ In addition, it seems that these drugs activate genes that have become abnormally silenced in cancer.^{69,70}

The question we face today in the epigenomics field applied to cancer research is ‘How to manage cancer with the new technologies and tools that are becoming available?’ The new technologies under development will facilitate the identification of better epigenetic markers and may aid in the development of more specific therapies. These drugs will be focused in a group of genes and not the entire epigenome (see Table 1 for epigenetic drugs). The other question is ‘What will be the impact of epigenomics in the clinics?’ In other words, how could we translate the discoveries from basic science to the patient’s bedside? In this regard, the FDA has already approved a few epigenetic

Table 4 Examples of companies offering predictive epigenetic-based tests for cancer diagnosis and monitoring

Company	Epigenetic test(s)	Impact for clinical decisions	Website
Oncomethylome sciences	<i>MGMT</i> methylation analysis	This test indicates if patients with unmethylated (functioning) <i>MGMT</i> will benefit from the addition of temozolomide to the standard treatment of radiation therapy. Patients with methylated (silenced) <i>MGMT</i> have longer progression-free and overall survivals with the combination of radiation therapy and temozolomide. This company also tests other genes for different tumor types in body fluids.	www.oncomethylome.com
Epigenomics AG	OncoSign and Epi proColon	Biomarker candidates are used to identify drug response in cancer patients based in the DNA methylation analyses of a set of genes. Other epigenetic tests are also offered for colon cancer management facilitating clinical decisions.	www.epigenomics.com
Sequenom	Cancer EpiPanels	The Cancer EpiPanel contains targets of more than 400 cancer-related genes. It includes prevalidated assays covering over 12,000 CpG sites in promoter regions of genes known to be involved in neoplastic transformation. The Cancer EpiPanel can be used to produce quantitative DNA methylation profiles helping in tumor classification.	www.sequenom.com
Exact sciences	Stool-based DNA methylation analyses	The company offers noninvasive, molecular screening technologies for the early detection of colorectal cancer using a combination of DNA methylation markers. Stool-based DNA technology is used for disease management.	www.exactsciences.com

drugs for different tumor types, and some of these are very promising (Table 1 and Figure 1). Molecular biomarkers, such as genes or groups of genes with changes in epigenetic modifications in tumors, have also been used to guide clinical decisions. Examples are epigenetic changes in *GSTP1* in prostate cancer, *p16ink4A* in different types of cancer, and *MGMT* in brain tumors (see Table 3). There are companies (ie, Oncomethylome Sciences, Epigenomics AG, Sequenom, and others) already offering a test with a panel of epigenetic markers covering different genomic regions for cancers (see Table 4 for more details). More recently, the company Exact Sciences released a combined test for four methylation markers for early detection of colon cancer with a 100% sensitivity. The advantages of using epigenetic markers for early detection of cancer and disease monitoring is that the test can be done in a small tumor sample or even in body fluids such as stool, blood, spinal fluid, and urine. Depending on the combination of markers obtained after the tests, clinicians are able to predict the appearance of the disease and also group cancer patients based in tumor aggressiveness and other clinical features, facilitating clinical decisions. Clearly, the impact of epigenomics in cancer management is expected to increase with the advent and development of new technologies.

Conclusions and future directions

In conclusion, the burgeoning fields of genomics and epigenomics comprise essential facets of modern cancer research. The FDA has already approved some epigenetic

drugs, and others are in clinical trials and under development, demonstrating that this field already affects the way we manage cancer. In addition, single genes and groups of genes from the same pathway have been identified as differentially methylated in cancers, and some have been used as molecular biomarkers in order to identify patients with a better or a worse prognosis. Histone modification changes have also been used as markers to monitor cancer patients. Clearly, epigenetic changes in tumors will affect the decisions that are made in the clinics for the patients, especially treatment regimens and disease progression monitoring. Future directions include the discovery of new biomarkers and the development of more efficient drugs against different tumor types with the evolving technologies and the emergence of a new generation of DNA sequencers. Based on the information discussed here, growing evidence indicates that new epigenomic tools will increasingly affect the way we monitor and manage cancer in the future.

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Disclosure

The author reports no conflicts of interest in this work.

Financial disclosure

The author is the founder of the web-based company Genomic Enterprise and works as a consultant (www.genomicenterprise.com). Genomic Enterprise has the purpose of ensuring the integration of genetic, epigenetic, and genomic information to interested customers. Genomic Enterprise's objectives include the development of business-to-business (B2B), business-to-consumer (B2C), and consumer-to-business (C2B) services in the scientific field.

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