INTRODUCTION

There is a basic need in medical science to assess the medical state of an individual. Doctors for millennia have practiced this principle including Greek doctors more than 2000 years ago that followed the teachings of Hippocrates of Cos [1]. Hippocratic textbooks advised doctors to examine their patient for “signs” to assist clinical decision making [2]. The principle of measuring a feature indicative of medical state to assist clinical decision making still forms a central role of current health services worldwide. The “measurable feature” is now referred to as a biomarker.

The term biomarker has been defined several times in the scientific literature as reviewed by Strimbu and Tavel [3], who have suggested the following as a definition of a biomarker: “The term ‘biomarker,’ a portmanteau of ‘biological marker,’ refers to a broad subcategory of medical signs – that is, objective indications of medical state observed from outside the patient—which can be measured accurately and reproducibly” [3]. This definition of a biomarker will be assumed for the purpose of this discussion.

Biomarkers are used routinely in a clinical setting to assess the medical state of patients and in several other medical contexts, including clinical trial endpoints, pharmaceutical development, and basic science research. A clinical example of a biomarker is plasma glucose. The World Health Organization (WHO) published its current diagnostic criteria for diabetes mellitus in 2006 using plasma glucose as a biomarker: fasted plasma glucose above 7.0 mmol/L (126 mg/dL) or 2 h post 75 g oral glucose challenge plasma glucose above 11.1 mmol/L (200 mg/dL) [4]. In the United Kingdom, the National Institute for Health and Care Excellence (NICE) recommended the WHO diagnostic criteria in their Type 2 Diabetes: The Management of Type 2 Diabetes guidelines published in 2009, updated in July 2014 [5], and they are currently being recommended for the National Health Service (NHS).
Biomarkers, therefore play a fundamental role in medical science today. With such an important role to play across huge fields, it is essential to ask the question: can current biomarkers be improved? In general, there is scope to improve current biomarkers by identifying biomarkers that arise earlier in the transition from normal physiology to a pathologic state, reducing the time it takes to generate results, and ensuring the results are more accurate, more reliable, and more individualized.

To highlight the potential for improvement in current biomarkers, type 2 diabetes mellitus (T2DM) will be discussed. The prevalence of T2DM is increasing globally; over the last three decades, it has more than doubled [6]. People with diabetes require at least two to three times the healthcare resources compared to people who do not have diabetes, and diabetes care may account for up to 15% of national health care budgets [7]. T2DM is a chronic disease characterized by reduced sensitivity to insulin, referred to as “insulin resistance.” Insulin resistance develops before changes in glycemic measurements such as increased glucose, glycated hemoglobin (HbA1c), and insulin and can be present more than a decade before diabetes [8]. Therefore, a simple blood test to detect insulin resistance before the onset of hyperglycemia would have an instant impact on clinical practice by enabling the identification of high-risk patients earlier in the transition to disease state and opens a window of opportunity to intervene and thus minimize the burden of disease. Progress is being made to identify early-onset biomarkers to reflect insulin resistance. A multiple linear regression algorithm of fasting blood levels of metabolites α-hydroxybutyrate, linoleoyl-glycerophosphocholine, oleate, and insulin that reflects insulin resistance has been developed [9]. Although this is a promising development toward earlier identification of insulin resistance, it can be improved.

There is a growing body of literature suggesting a role for epigenetic factors (changes in DNA regulation and subsequent gene expression) as a molecular link between environmental factors and T2DM [10]. Furthermore, a recent study has suggested some epigenetic changes that may be involved in the progression of diabetes and/or that the development of complications may be apparent at the prediabetes state or during the transition to diabetes [11]. Therefore, epigenetic changes themselves can be used as biomarkers to indicate medical state. Although T2DM has been used as an example here, epigenetic modifications are applicable to several other diseases, including cancer. With advances in technology to allow timely measurement of epigenetic changes, epigenetic biomarkers hold potential to improve a range of biomarkers and thus improve medical sciences.

WHAT ARE EPIGENETIC BIOMARKERS?

The term epigenetics was coined by C. Waddington in 1942 when he was investigating the causality between the genotype and the phenotype [12]. The epigenome refers to the complete description of all potentially heritable modifications of the genome without any changes in primary DNA sequences [13]. The epigenome therefore comprises all changes in gene regulation via modification of the genome, including pathologic changes that can lead to disease states. Epigenetic alterations are necessary in several regulated cell processes such as differentiation and proliferation, however they, like gene mutations, can contribute toward the pathogenesis and molecular heterogeneity of several diseases.

There are several levels of epigenetic modifications ranging from direct DNA modification to higher orders of DNA organizational structure changes and also the influence of noncoding RNAs. Measuring these changes within the genome provides an opportunity to assess the regulation of DNA. These epigenetic modifications can be measured accurately and reproducibly and fulfill the definition of a biomarker. Therefore, epigenetic biomarkers can be broadly defined as measurable modifications of the genome with preserved DNA sequence. Epigenetic modification types that have been shown to play a role in pathogenesis are discussed later in hierarchical level from direct DNA modifications to histone and chromatin alterations and finally noncoding RNAs. This approach to discuss epigenetic modifications is for clarity, although emerging evidence suggests extensive crosstalk between different types of epigenetic modification such as DNA and histone methylation [14] (Fig. 10.1).

DNA Methylation

The best-characterized epigenetic modification in mammals is DNA methylation, a covalent addition of a methyl group at carbon five primarily to cytosine (C) bases within cytosine–guanine (CpG) dinucleotides; non-CpG methylation is observed only in stem cells in the body of actively transcribed genes [15]. CpG dinucleotide sequences are enriched in CpG islands. Takai and Jones used the complete genomic sequences of human chromosomes 21 and 22 to examine the properties of CpG islands and derived an updated definition of CpG islands as “regions of DNA of greater than 500 bp with a G + C equal to or greater than 55%” [16]. DNA methyltransferases (DNMTs) are a family of enzymes that regulate the methylation state of DNA. DNMT3a and DNMT3b catalyze de novo DNA methylation whereas DNMT1 is responsible for the maintenance of existing DNA methylation. DNA methylation is copied to the new strand during DNA replication and is therefore heritable through mitosis and represents a mechanism of cellular memory of gene expression states in previous parental cells.
DNA methylation, though not found in all organisms, is highly correlated with gene silencing [17]. However, the effect of methylation depends on the context of where the methylation occurs in the genome. GpG islands exist mainly in the promoter region of genes and have been shown to be abnormally methylated in cancer cells [18]. Promoter region CpG island methylation is correlated with transcriptional silencing; however, methylation that occurs in CpG islands in sites outside the promoter region, known as gene body methylation, has been associated with transcriptional activation [19]. In the process of tumor formation, hypermethylation in the CpG islands of gene promoters and demethylation of the entire genome occur simultaneously. Widespread hypomethylation has also been linked to tumor formation via a change of chromatin structure, lower levels of condensed chromatin, causing an increase of genome instability, leading to the increased occurrence of tumors. For example, microsatellite DNA sequences are more easily mutated when they are hypomethylated and have been identified in several kinds of tumor models [20]. Furthermore, hypermethylation in the CpG islands of promoter regions and the resulting suppression of tumor suppressor genes also contributes to tumor developments [21]. Therefore, DNA methylation biomarkers must be carefully selected at specific locations within the genome to provide meaningful information about the risk of developing disease.

**Histone Modification**

Histone modification is a further epigenetic mechanism and refers to the alteration of histone tails via methylation, acetylation, phosphorylation, ubiquitination, and sumoylation [22]. DNA is wrapped around an octamer formed from two subunits of each histone protein—H2A, H2B, H3, and H4—to make a nucleosome, the basic unit of chromatin. Histone proteins are prone to undergo posttranslational modifications because of their flexible charged NH$_2$ terminus, known as the histone tail, that protrudes from the globular domain. Histone modifications alter the interaction of DNA with histones and consequently alter the accessibility of DNA transcription sites to transcription factors or RNA polymerase II. Therefore, posttranslational modifications to histone tails influence the structural state of chromatin and the subsequent transcriptional status of genes within particular locations.

Histone methylation normally occurs on lysine and arginine residues of histones H3 and H4 and is controlled by histone methyltransferases (HMTs) and histone demethylases (HDMs). Lysine residues may present different methylation states with the addition of one, two, or three methyl groups, leading to various levels of DNA accessibility. Like DNA methylation, the effect of methylation depends on the specific context and location. For example, trimethylation at H3K27, H3K9, and H4K20 and dimethylation at H3K9 closes chromatin, whereas trimethylation at H3K4 and H3K36 opens chromatin [23]. Histone acetylation is regulated by histone acetyltransferases (HATs) that covalently add an acetyl group to lysine residues and histone deacetylases (HDACs) that remove an acetyl group. HATs promote transcription by neutralizing a positive charge, resulting in opening of chromatin and transactivation of specific genes, whereas HDACs generally result in transcriptional inactivation of the involved DNA due to chromatin condensation [24]. Aberrant gene silencing and tumorigenesis has been strongly implicated to deregulated HDAC activity in human cancers [25]. Collectively, several combinations of histone modifications at specific locations of the genome can contribute to a more accessible or inaccessible
chromatin structure and consequent activation or repression of gene expression. Therefore, assessment of histone modification biomarkers at certain genome locations may provide insight into the likelihood of developing a particular disease.

**Chromatin Remodeling**

Nucleosome packaging of DNA condenses and organizes the genome; however, it obstructs several regulatory elements. To enable dynamic access to packaged DNA and to adapt nucleosome composition in chromosomal regions, cells have evolved a set of chromatin remodeling complexes. These complexes use the energy of ATP hydrolysis to destabilize, move, eject, or restructure nucleosomes [26]. Mutations in particular subunits of a chromatin remodeler can lead to specific types of cancers, suggesting that certain subunits contribute to distinct transcriptional programs that regulate growth or differentiation. For example, the regulator subunit hSNF5/INI1 plays a critical role in cell cycle regulation and tumor suppression, and biallelic loss of Snf5 is a constant feature of aggressive human malignant rhabdoid tumors (MRTs) [27]. Therefore, screening for particular chromatin-remodeling biomarkers may present an ideal approach to assess the likelihood of developing disease.

**Noncoding RNAs**

Regulatory noncoding RNAs (ncRNA) can be categorized based on their length, and the most studied class of ncRNAs is microRNAs (miRNA), approximately 22 nucleotides in length and responsible for repression of more than half of protein-coding genes by controlling the translation of messenger RNA (mRNA) into proteins [28]. miRNAs play a role in translational repression by incomplete pairing with the 3' untranslated region (3'UTR) of target genes or through cleavage of mRNA. miRNAs are involved in the regulation of several biological processes including cell differentiation, proliferation, and apoptosis [29]. Abnormal expression of miRNAs has been linked to different cancers and now is being used to classify many human cancers [30]. Therefore, certain miRNAs may present as ideal biomarkers for disease.

**METHODS FOR ASSESSING EPIGENETIC CHANGES FOR BIOMARKERS**

As the association between gene expression and DNA methylation, histone modifications, and chromatin remodeling is clarified, a valuable aspect to consider is how the epigenetic status coordinates gene expression to enable cellular processes such as differentiation and, furthermore, how these vital epigenetic processes may be aberrant in pathological conditions. In order to investigate these and other epigenetic-related phenomena, robust and reliable technologies for measuring epigenetic changes are essential. A key advantage of epigenetic biomarkers over other markers is the availability of technologies for detecting biomarkers in tissue samples and biofluids [31]. Current techniques for investigating a range of epigenetic modifications have been developed over the last 40 years and build on existing technologies such as microarray and pyrosequencing. Ladd-Acosta has summarized an overview of epigenetic measurement tools in Table 10.1 of their recent review [32]. The progression of technological advancement has enabled routine and straightforward sample processing to be used to obtain novel epigenetic modification status information. Several different epigenetic analysis technologies are commonplace in today’s epigenetic research boom; however, a few key principles and techniques are detailed later in the chapter to demonstrate the fundamental aspects of measuring epigenetic changes.

**Genomewide Epigenetic Analysis**

Genomewide methylation analysis permits the analysis of global epigenetic changes and therefore the opportunity to assess the whole genome. The first genomewide, single-base resolution maps of methylated cytosines in a mammalian genome were published in 2009 [33]. There are different approaches for genomewide methylation analysis as discussed later in the chapter.

Microarrays can be used to investigate genomewide changes in all epigenetic modifications. Microarray technology has permitted high-throughput investigations of biological materials for molecular-level analysis with regard to physiology and disease. Based on PubMed and GEO submissions, the microarray platform of choice for epigenome-wide association studies is the Infinium HumanMethylation450 BeadChip [34]. It provides an assessment for more than 480,000 CpG loci, covering key features of the human genome including CpG islands, shores, and shelves as well as promoters, gene bodies, intergenic, and imprinted regions [35]. Large volumes of data are generated from microarrays that requires vigilant analysis to gain insights into the material assayed, which then must be validated using a different method before examination can continue. An integrated analysis pipeline has been designed to assist normalization and processing of high-throughput
DNA methylation analysis [36]. Furthermore, software tools for large-scale analysis and interpretation of DNA methylation data have been developed [37]. Methylation alterations of CpG islands in ovarian tumors have been profiled by microarray analysis to identify candidate biomarkers for diagnosis and prognosis of the disease [38].

miRNA profiling is a further example of genomewide epigenetic analysis. There are several miRNA-profiling techniques presently employed including quantitative PCR (qPCR), hybridization, and next-generation sequencing [39]. Next-generation sequencing is anticipated to be the future gold standard for miRNA profiling because of its quantitative ability; however, currently it is a relatively undeveloped technique and is more expensive than other alternative methods. miRNA qPCR arrays utilize existing qPCR methodology with adaptations to form a semiquantitative, practical, and quick method to screen for miRNAs. Multiple miRNAs can be compared in a single real-time qPCR (RT-qPCR) reaction. An example of a commonly used miRNA profiling method is the TaqMan Array MicroRNA. This method employs two steps to relative quantification of miRNA expression. First, a multiplex reverse-transcription PCR with a pool of stem loop primers is used to yield miRNA-specific cDNA from a total RNA sample. Second, a qRT-PCR reaction is carried out with the cDNA from the initial PCR in separate wells of a PCR plate. TaqMan supplies miRNA plates with 384 wells each with a separate specific forward primer, reverse primer, and a TaqMan probe for an miRNA. During the qRT-PCR reaction, the fluorescence emitted during DNA amplification is plotted against the PCR cycle number. A threshold value is used to find the exponential phase of the reaction and determine the cycle number when the gene is expressed. qRT-PCR miRNA arrays are used to find the expression fold difference between samples using the comparative delta Ct method. Microarray analysis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Tissue</th>
<th>Epigenetic Change</th>
<th>Genome Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>Various</td>
<td>Various</td>
<td>DNA CpG hypomethylation</td>
<td>[11]</td>
</tr>
<tr>
<td>Various cancers</td>
<td>Various</td>
<td>Promoter region CpG island hypermethylation</td>
<td>Gene silencing</td>
<td>[21,22,24]</td>
</tr>
<tr>
<td>Various cancers</td>
<td>Various</td>
<td>Global hypomethylation</td>
<td>Gene expression disruption</td>
<td>[22,23]</td>
</tr>
<tr>
<td>Various cancers</td>
<td>Various</td>
<td>Deregulated HDAC</td>
<td>Gene silencing</td>
<td>[28]</td>
</tr>
<tr>
<td>Various cancers</td>
<td>Various</td>
<td>Aberrant miRNA expression</td>
<td>Gene expression disruption</td>
<td>[34]</td>
</tr>
<tr>
<td>Malignant rhabdoid tumors</td>
<td>Various</td>
<td>Chromatin regulator subunit dysfunction</td>
<td>Chromatin remodeling defect</td>
<td>[30,31]</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Ovaries</td>
<td>DNA CpG methylation</td>
<td>Gene expression disruption</td>
<td>[37]</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>Squamous cells</td>
<td>DNA CpG methylation</td>
<td>Gene expression disruption</td>
<td>[39]</td>
</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>Various</td>
<td>DNA CpG methylation</td>
<td>Gene expression disruption</td>
<td>[42]</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Liver</td>
<td>Aberrant miRNA expression</td>
<td>Gene expression disruption</td>
<td>[47]</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Colon</td>
<td>DNA CpG hypermethylation</td>
<td>Gene silencing</td>
<td>[50,53]</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Colon</td>
<td>miR-137 methylation</td>
<td>Gene silencing</td>
<td>[54]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Prostate</td>
<td>Various</td>
<td>Gene expression disruption</td>
<td>[57,61]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Prostate</td>
<td>Global DNA CpG hypomethylation</td>
<td>Gene expression disruption</td>
<td>[58,59]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Prostate</td>
<td>DNA CpG hypermethylation in GSTP1</td>
<td>Gene silencing</td>
<td>[60]</td>
</tr>
<tr>
<td>Esophageal adenocarcinoma</td>
<td>Esophagus</td>
<td>Various</td>
<td>Gene expression disruption</td>
<td>[66]</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Stomach</td>
<td>Aberrant miRNA expression</td>
<td>Gene expression disruption</td>
<td>[68]</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>Kidney</td>
<td>Various</td>
<td>Gene expression disruption</td>
<td>[69]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Breast</td>
<td>DNA CpG methylation</td>
<td>Gene expression disruption</td>
<td>[70,72]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Breast</td>
<td>Histone methylation</td>
<td>Gene expression disruption</td>
<td>[86,87]</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>Brain</td>
<td>DNA CpG methylation</td>
<td>Gene expression disruption</td>
<td>[74,75]</td>
</tr>
</tbody>
</table>
for miRNA has been conducted to compare hepatocellular carcinoma and nontumorous tissues and the results used to generate an algorithm with a prediction accuracy of 97.8% [40]. Therefore, miRNA arrays have the potential for epigenetic biomarker screening applications.

**Locus-Specific Epigenetic Analysis**

Pyrosequencing is a locus-specific technique; it can be used to quantify DNA methylation at specific GpC sites within the target region of interest. It is essential to bisulfite-convert the DNA sample prior to pyrosequencing for DNA methylation quantification to allow detection of methylation sites. During bisulfite conversion, double-stranded DNA (dsDNA) is denatured and methyl cytosines are unchanged, whereas unmethylated cytosines are deaminated to form a cytosine-sulfonate derivative, which is converted to a uracil-sulfonate derivative and finally a uracil base [41]. During subsequent polymerase chain reaction (PCR) amplification, methylated cytosines are amplified as cytosines whereas the uracils are amplified as thymines. During pyrosequencing, single nucleotides are released and incorporated if complementarily into the sequence, a pyrophosphate (PPI) is released as a by-product and a cascade reaction takes place. The PPI is converted to ATP in the presence of adenosine 5’-phosphosulfate by the enzyme ATP sulfurylase. Luciferase is driven by the ATP to convert luciferin to oxyluciferin, which generates visible light proportional to the amount of ATP, and thus proportional to the number of dispensed bases and incorporated nucleotides. A charge-coupled device detects the visible light and records the output. Pyrosequencing is already used in the clinic for prognostic purposes for newly diagnosed glioblastoma, although some uncertainties remain regarding methodologies, cut-off definitions, and optimal use in the clinical setting [42].

**DIAGNOSTIC EPIGENETIC BIOMARKERS**

**Early Clinical Diagnosis**

Clinical outcomes for patients are improved with early diagnosis of disease. For example, population screening for cancers and the surveillance of high-risk patients allows the early diagnosis of cancer before extensive local tumor development and metastasis. This reduces morbidity to the patient by allowing less invasive treatment, resulting in fewer complications and side effects. However, most of the current investigation methods used for cancer screening, such as histologic assessment, radiologic procedures, and endoscopic evaluations have a limited specificity and sensitivity, can be costly, and suffer with poor patient compliance. Furthermore, screening for most diseases across whole populations is not routine; therefore, diseases are only diagnosed when patients become symptomatic, by which time the patient may have had the disease for considerable time.

Consequently, there is a need for a minimally invasive assessment that could be used in conjunction with existing methods to improve screening sensitivity and specificity and be sufficiently cost-effective to be used for several diseases. Epigenetic alterations such as histone modification or promoter hypermethylation occur early in transition to a disease state; therefore, they can be used to indicate early transition to a pathological state [43]. In addition, the technology to assess epigenetic changes is already in use (see previous section). Hence measuring epigenetic biomarkers is a promising tool for the early diagnosis of disease. Although there are many other diseases that can benefit from measuring early epigenetic biomarker changes, the potential diagnostic biomarkers for colon and prostate cancer are discussed as examples because they have the potential to significantly improve existing clinical practice.

**Early Colon Cancer Diagnosis**

Colorectal cancer is the second leading cause of cancer-related deaths in the United States [44]. Screening can help to reduce the burden of disease; for example, a study with more than 150,000 subjects found that screening with flexible sigmoidoscopy with a positive result if a polyp or mass was detected was associated with a significant decrease in colorectal cancer incidence and mortality [45]. If a screening test can diagnose colon cancer before the development of a polyp or mass, it would allow earlier intervention and benefit patient outcome. Epigenetic biomarkers have the potential to help early diagnosis of colon cancer.

Gene silencing by DNA methylation in colorectal cancers has been reported by several studies [46]. These epigenetic modifications play a role in the transformation of a cell, particularly if the changes alter genes that play a role in maintaining genomic stability. Aberrant crypt foci (ACF) are colon surface abnormalities and are used as biomarkers of increased risk of colon cancer, heightened if dysplasia is also present [47]. ACF promoter hypermethylation has been recorded in the DNA repair genes *hMLH1* and *MGMT*. In all the lesions when *MGMT* was hypermethylated, a microsatellite instability
(MSI)–low phenotype was found, suggesting that the lesions may be precursors to MSI-low colorectal cancer [48]. Furthermore, genomic instability, DNA methylation changes, and loss of heterozygosity found in ACF indicate that DNA methylation changes can be used as an epigenetic biomarker of early colon tumor development. Assessment of hMLH1 and MGMT promoter methylation status from a colon biopsy during colonoscopy screening may indicate likelihood of colon cancer development and has potential to be used in combination with existing diagnosis methods; however, further investigations linking the gene modifications to tumor development are required. The promoter methylation of hMLH1 and MGMT has been measured in different segments of the colon in healthy men and women undergoing screening colonoscopy. Promoter methylation varied in different segments and by subject age with most hypermethylation in the right colon, consistent with colorectal cancers; therefore, the epigenetic changes may be important aspects of the early carcinogenic process [49]. A distinct epigenetic modification that has been implicated with early stages of colorectal cancer is the methylation of miR-137. Normal colon mucosa expressed miR-137, and it was inversely correlated with methylation level, and therefore it has been suggested to act like a tumor suppressor in the colon [50]. Therefore, the methylation and consequent silencing of miR-137 in colorectal cancers could be used as a biomarker for early tumorigenesis. A recent review of molecular phenotypes of colorectal cancer and potential clinical applications has been published and includes the CpG island methylator phenotype (CIMP) [51]. The FDA has already approved epigenetic markers for colorectal cancer, for example, SEPT9 (ColoVantage) and vimentin (ColoSure) [52].

Early Prostate Cancer Diagnosis

Serum prostate-specific antigen (PSA) level is the only biomarker currently used for the detection and monitoring of prostate cancer. However, PSA has attracted criticism for use as a biomarker for several reasons. There are no definitive cut-off PSA levels that define prostate cancer; some men have high PSA without prostate cancer [53], whereas others with prostate cancer have low PSA [54]. In addition, PSA is not specific to prostate cancer, and serum PSA cannot predict severity of prostate cancer or likelihood of progression. Therefore, improved biomarkers for prostate cancer may hold significant clinical potential. Epigenetic modifications are common in prostate cancer and are thought to contribute both to disease initiation and progression [55]. The mechanisms for prostate cancer epigenetic modifications are not fully elucidated; however, the fact that they occur much more frequently than mutations and are common in premalignant stages of the disease make them appealing for diagnosis.

DNA methylation is the most studied epigenetic modification in prostate cancer. The use of DNA methylation based biomarkers for prostate cancer is attractive because of the high stability of DNA, straightforward analysis with existing techniques, and current evidence suggesting global hypomethylation is associated with advanced metastatic stage. Human prostate tumor tissues have a significant decrease in the global levels of methylated cytosine in patients with recurrent prostate cancer compared to patients without recurrence [56]. However, no association has been found between global DNA methylation and prostate cancer patient survival [57]. More recent studies have focused on specific gene hypermethylation and resulting gene silencing. In prostate cancer, several specific hypermethylated genes have been identified. Of the extensive list of hypermethylated genes associated with prostate cancer, the most established biomarker is DNA methylation of the glutathione-S-transferase P1 (GSTP1) gene. DNA methylation of GSTP1 is an attractive biomarker for prostate cancer because it has a high specificity (>90 %) compared to serum PSA (20%) [58], the level of promoter methylation can differentiate prostate cancer from other prostatic diseases and are associated with different stages of prostate cancer, and it can be easily measured with a noninvasive procedure in serum plasma or urine. Although GSTP1 has a much higher specificity than serum PSA, it is not 100% specific to prostate cancer because it is also hypermethylated in some other cancers. To improve the specificity and sensitivity, the methylation profile of several genes can be assessed in combination, or in combination with histone modifications and miRNA screening and considered in addition to gene mutations [59]. Taken together, there is growing evidence that epigenetic biomarkers for the early detection of prostate cancer can provide a viable step forward for improving clinical outcomes. A DNA methylation test for prostate cancer (ProCaM) has been approved by the FDA and is discussed in a recent review of diagnostic and prognostic epigenetic biomarkers in cancer [60].

EPIGENETIC BIOMARKERS FOR RISK STRATIFICATION

The provision of healthcare in developed countries is being challenged by several factors. These populations are suffering more chronic illness in aging populations, while simultaneously the hopes and expectations of patients are increasing and healthcare budgets are coming under pressure. Healthcare costs for populations are not equally attributed to each member of the population; indeed, there are minorities of patients who account for a disproportionally high fraction of the costs [61].
If these patients are identified earlier and offered earlier support and care, then they may achieve better health outcomes and experience of care while also reducing healthcare costs from avoided complications and potential hospital admissions.

Several health care organizations are employing risk stratification to aid selection of care in order to combat the challenges of limited resources. Risk stratification can be broadly defined as estimating the probability of an outcome and then grouping the results into risk strata. In a medical context, risk stratification can refer to several situations, including the likelihood of developing disease, the likelihood of progression of a disease, or the likelihood of responding to certain therapies. Risk stratification to identify patients that have a high risk of an adverse effect is analogous to population screening; the objective is to find patients where further tests or treatment will likely provide benefits that outweigh harms. The overall aim for risk stratification for the population is to ensure that the benefits outweigh the costs.

Risk stratification has great potential to assist healthcare systems, however it also has a range of potential issues. It is vital to consider that, like screening tests, no risk stratification process is completely accurate. If there is a false-positive result, the demand on resources increases and there is considerable morbidity to the patient. Equally, a false negative (poor sensitivity) result can reassure both the patient and doctor that there is no disease process going on when in fact there is. Thus, it is important to realize the limitations of risk stratification while balancing limited healthcare resources.

Epigenetic biomarkers have the potential to be used for future risk stratification schemes. The technology to identify epigenetic biomarkers is already available (discussed in an earlier section). Epigenetic modifications occur early in the transition to a disease state so they can be used to detect patients who are at high risk of developing disease [11]. Epigenetic biomarkers can be measured in easily accessible biological fluids [31]. When more than one epigenetic modification, such as gene promoter hypermethylation, is grouped to determine risk, the outcome is reliable [62]. Therefore, screening tests for certain epigenetic changes may present a way of stratifying risk for developing disease.

**Risk Stratification for Developing Disease**

The quality of a single genotype to produce different phenotypes in response to differing environments is termed “plasticity.” Maximal plasticity is thought to occur during development. Plasticity during development attempts to modify gene expression such that a phenotype is produced that will best suit the predicted later environment [63]. The resulting phenotype is more likely to remain healthy when the phenotype matches the predicted environment. However, when there is a disparity between phenotype and environment, the ability of the individual to respond to environmental challenges is compromised. Therefore, the extent of mismatch indicates the susceptibility of an individual to chronic disease [64]. There is a large body of evidence that suggests epigenetic modifications mediate developmental plasticity and can span generations to influence the development and behavior of subsequent generations [65]. The concept that events early in life affect long-term health has been termed the “developmental origins of health and disease” (DOHaD) hypothesis [65]. Despite the collective knowledge that developmental epigenetic changes alter phenotype, the exact genes that are altered for specific susceptibility to certain diseases remain elusive. The identification of such modifications remains the center of intense investigation. Once these epigenetic changes are identified and verified, they can form the basis of screening tests to assess risk of developing diseases. A simple blood test could be developed to assess the methylation status of a defined set of genes and analyzed to determine risk of developing a particular disease or several diseases.

**Risk Stratification for Disease Progression**

Healthcare systems with limited resources are also faced with the issue of allocating their resources in a way to fairly and maximally benefit the health of the population. Diseases vary in severity, often referred to as stages, where the least adverse may have minor symptoms such as altered histology and more severe stages may have debilitating conditions and comorbidities that can ultimately be life-threatening. This transition through disease state is termed “progression.” Progression is not inevitable, and the rate of progression is inconsistent. Therefore, if a test could assess the risk of progression, then healthcare resources could be directed to patients who are identified as high-risk. Because epigenetic modifications change during the progression of disease, measuring specific relevant epigenetic modifications may indicate risk of progressing to a more severe disease state and therefore assist clinical decision making.

For example, Barrett esophagus predisposes to esophageal adenocarcinoma; however, there is a low incidence of esophageal adenocarcinoma in Barrett esophagus [66]. Therefore, endoscopic surveillance is inefficient for assessing disease progression. A three-tiered risk stratification strategy has been developed based on systematically selected epigenetic and clinical parameters to improve Barrett esophagus surveillance efficiency [66]. This will allow risk stratification of patients and assist clinical decision making in the management of patients with Barrett esophagus. It is useful to know the risk of disease progression in colorectal cancer to help inform clinical decision making. The presence of an epigenetic defect in the colon mucosa around the neoplastic lesion may indicate a higher risk of metachronous neoplastic lesions and could help to...
identify which patients require more radical surgery [67]. This concept is termed “field cancerization theory” and has been supported by several reports in the literature. Field cancerization is not restricted to colon cancer; indeed, epigenetic changes have been identified in gastric tumor adjacent tissues by high-throughput miRNA sequencing [68]. Further epigenetic field studies are required to help understand why multiple primary tumors and locally recurrent cancer occurs. This information will be extremely useful for risk stratification and help healthcare systems to optimally allocate resources. Epigenetic mechanisms involved in diabetic nephropathy progression have been identified [69]. And a recent study suggests that selective targeting of DNA methylation changes during breast cancer progression [70]. With this information, a risk stratification tool can be designed to estimate a patient’s risk of progressing to more severe renal dysfunction or breast cancer. Furthermore, epigenetic biomarkers can be used to assess the risk of latent viral associated diseases developing. For example, Epstein-Barr virus–associated gastric carcinoma (EBVaGC) is a distinct subtype that accounts for nearly 10% of gastric carcinomas. Global CpG island hypermethylation, which induces epigenetic silencing of tumor suppressor genes, is a unique feature of EBVaGC [71]. Therefore, assessing a patient’s global CpG island hypermethylation may be a way to assess their risk for developing EBVaGC and helping inform their clinical management. The technique of measuring epigenetic biomarkers to enable risk stratification for progression of disease can be applied to any disease with an epigenetic signature. Further research is needed to build on current knowledge and assess the epigenetic changes during the progression of other diseases to build a comprehensive database to assist the development of epigenetic biomarker risk stratification tests.

## Risk Stratification to Assess Therapeutic Response

Cellular heterogeneity exists within diseases [72]. For example, hepatocytes in hepatitis, epithelial cells in renal dysfunction, pancreatic beta cells in diabetes, and tumor cells in cancer. The underlying mechanisms for this are in part due to epigenetic modifications. The cellular heterogeneity may explain why existing therapies have varying degrees of efficacy. Knowledge of the epigenomic state of a particular gene or cluster of genes may help determine the best therapy or combination of therapies to use. For example, if after DNA methylation analysis, a tumor suppressor gene promoter region is hypermethylated and silenced, then it can be targeted site specifically with DNMT1 inhibitors and in combination with agents to remove methyl groups from cytosine bases with the aim to reactivate the previously silenced gene. Conversely, if an oncogene is hypomethylated in its promoter region and the gene product is highly expressed, the specific promoter region can be targeted with DNMT3a and DNMT3b agonists to induce methylation and gene silencing. In order to achieve site-specific targeting in mammalian genomes, clustered regularly interspaced short palindromic repeats (CRISPR) technology can be modified by excluding the Cas9 endonuclease protein to direct treatment to a certain location within the genome without causing any alteration to DNA sequence [73]. An example of successful epigenetic stratification for therapeutic intervention is in the treatment of glioblastoma by the alkylating agent temozolomide. O6-methylguanine–DNA methyltransferase (MGMT) is a repair enzyme that repairs the DNA lesion caused by alkylating agents, thus preventing the induction of apoptosis [74]. Methylation and loss of expression of MGMT results in less DNA repair and increases the sensitivity of cells to temozolomide. Evidence that MGMT promoter methylation is a valid predictive biomarker for radiotherapy rather than alkylating agent chemotherapy has come from prospective randomized trials of elderly patients with glioblastoma [75] (Table 10.2).

<table>
<thead>
<tr>
<th>Epigenetic Change</th>
<th>Altered Genes or Targets</th>
<th>Genome Effect</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CpG island hypomethylation</td>
<td>Global, genomewide</td>
<td>Gene dysregulation</td>
<td>Recurrent prostate cancer</td>
<td>[55]</td>
</tr>
<tr>
<td>DNA hypermethylation</td>
<td>HPP1, p16, RUNX3</td>
<td>Gene silencing</td>
<td>Esophageal adenocarcinoma</td>
<td>[65]</td>
</tr>
<tr>
<td>Methylation of miRNA locus</td>
<td>EVL/hsa-miR-342</td>
<td>Gene silencing</td>
<td>Colorectal cancer</td>
<td>[66]</td>
</tr>
<tr>
<td>DNA hypermethylation</td>
<td>hsa-miR-3131, hsa-miR-664,</td>
<td>Gene silencing</td>
<td>Gastric cancer</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>hsa-miR-483, hsa-miR-150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA hypermethylation</td>
<td>XIST</td>
<td>Gene silencing</td>
<td>Breast cancer</td>
<td>[69]</td>
</tr>
<tr>
<td>GpG island hypermethylation</td>
<td>Global, genomewide (tumor suppressor genes)</td>
<td>Gene silencing</td>
<td>Gastric cancer</td>
<td>[70]</td>
</tr>
<tr>
<td>DNA hypermethylation</td>
<td>MGMT</td>
<td>Gene silencing</td>
<td>Glioblastoma</td>
<td>[75]</td>
</tr>
</tbody>
</table>

Epigenetic biomarkers with the potential to be used in risk stratification programs for various diseases.
EPIGENETIC BIOMARKERS FOR CHEMOPREVENTION

What Is Chemoprevention?

Healthcare systems with limited resources must maximize the health gains of the population. A strategy currently employed by healthcare systems to help reduce the burden of disease is to administer medication or treatment to prevent a disease from occurring. This concept is termed “chemoprevention” and the medication administered is termed “prophylactic.” Vaccinations are examples of extremely useful prophylactics and successful form of chemoprevention. Vaccinations are currently limited to communicable diseases, where the disease can be transmitted from one sufferer to another, so the disease is contagious or infectious. Current healthcare systems perform risk stratification to decide which subsets of the population to recommend vaccination for. If the transmission of communicable disease is blocked by sufficiently immunized individuals, then “herd immunity” is achieved. The entire population does not require vaccination to achieve herd immunity. Therefore, it is cost effective to ensure only a sufficient percentage of the high-risk population to achieve herd immunity is vaccinated. For example, to prevent the burden of disease caused by tuberculosis (TB) in England, the National Health Service (NHS) administers the Bacillus Calmette-Guerin (BCG) vaccination to anyone under the age of 35 years [76]. However, before the BCG vaccination is administered, a tuberculin skin test is performed in certain high-risk individuals to assess whether the individual has been infected with or has active TB [76].

Chemoprevention for Noncommunicable Diseases

Developed countries are facing challenges with aging populations and considerable noncommunicable chronic disease. If individuals who are at a high risk of developing chronic disease can be identified early, a window of opportunity is opened where they can be offered prophylactic medication to minimize their chance of developing disease. The search for ideal agents for cancer chemoprevention has been taking place over the last three decades and some promising advancements have been made [77]. Further research is needed to identify ideal chemopreventive modulators for other diseases. Chemopreventive intervention is best suited to a defined population that is at a relatively higher risk for developing the disease with the aim to slow down, stop or reverse the progression of the disease [78]. Therefore, it is necessary to identify individuals who are at a higher risk of developing disease. The process of administering chemopreventive healthcare to certain high-risk individuals requires risk stratification and is analogous to immunizing high-risk individuals. Risk stratification to identify high-risk individuals can be achieved with epigenetic biomarker screening (see previous section). In this instance, the epigenetic biomarker screening to identify whether chemopreventive medication should be administered is analogous to the tuberculin skin test prior to BCG vaccination. This type of risk stratification screening will allow healthcare systems to optimally distribute resources to maximally benefit the individual and population as a whole. A patient can have their epigenome analyzed for specific epigenetic changes, such as methylation status in certain genes or expression of certain miRNAs. A high-risk profile for a specific disease can be detected, and so the patient can be offered preventative therapy to minimize his or her risk of developing the disease. The potential benefits of chemopreventive medication must outweigh the harms; therefore, careful analysis of the efficacy of the treatment must be weighed up against potential side effects.

Epigenetic Biomarker Analysis to Assist Chemopreventive Medication

There are several examples where epigenetic biomarker analysis can assist delivery of chemopreventive medication. Following the field cancerization theory, individuals at high risk of malignancy could be identified based on epigenetic biomarker field defects in their tumor adjacent tissues [67]. Pharmacological therapy could then be developed to target the aberrant epigenetic status, signaling pathway or multiple pathways with the aim to modify the field change in order to reduce the risk of subsequent malignant transformation. This may restrict the tumor to a benign state and may reduce or eliminate the need for surgical intervention. By preventing metastasis and reducing the potential side effects associated with surgery, the chemoprevention will increase the survival chances for the patient. Patients with Barrett esophagus can be stratified based on systematically selected epigenetic and clinical parameters [66]. The patients with a high-risk profile can be offered chemopreventive medication to limit the transformation to esophageal adenocarcinoma.

Epigenetic mechanisms involved in diabetic nephropathy progression have been identified [69]. Therefore, patients with diabetic nephropathy can have an epigenetic biomarker screen to assess their risk of progressing toward end-stage renal failure. Individuals with a high risk following epigenetic analysis can then be offered preventative medication. This might prolong renal function sufficiently to avert the need for more severe treatment like renal replacement therapy and therefore improve the individual’s quality of life, extend their life-years gained while also reducing the healthcare cost of renal replacement therapy. The extent of DNA methylation in clusters of genes involved in breast cancer has been shown to
change during breast cancer progression [70]. Therefore, DNA methylation screening at the specific gene clusters will enable an estimate of breast cancer stage and potentially predict risk of progression. Individuals at a high risk for progression can be offered chemopreventive medication with the aim to minimize disease progression.

The technique of measuring epigenetic biomarkers to enable risk stratification to assist chemoprevention clinical decision making can be adapted to any disease with a well-defined epigenetic profile. However, caution should be applied when assessing epigenetic biomarkers because of their dynamic nature. This reinforces the need for continued research to build on existing knowledge of epigenetic changes during the progression of diseases. Ideally, a comprehensive database of epigenetic modifications during disease progression should be compiled to assist the development of epigenetic biomarker risk stratification tests.

**THERAPEUTIC IMPLICATIONS OF EPIGENETIC BIOMARKERS**

The existence of differential epigenetic modifications associated with disease progression has important clinical implications for the development of strategies to assess the effectiveness of therapies. Being able to assess the effectiveness of therapy will help the development of therapeutics and assist clinical decision making to allow best allocation of resources. For instance, a patient with a diagnosed disease state can react differently to chemotherapy compared to other patients diagnosed with the same diagnosis. To help understand why the patients react differently to chemotherapy and to help decide which therapies they may respond to, patients can have their epigenetic profile analyzed. Based on this information, an informed decision can be made about the management of the disease.

**Epigenetic Plasticity as a Therapeutic Target**

The plasticity between cellular phenotypes in disease states presents considerable challenges to therapy [79]. At the onset of several diseases, a physiologically regulated and healthy cell undergoes a transition toward a suboptimal state. This transition is mediated by epigenetic alterations regulated by the cellular microenvironment through cytokine and chemokine signaling, transcriptional regulation, including differential expression of miRNAs, or a combination of these [80]. Although plasticity between different cell types is fundamental to normal biology in development and adult homeostasis to appropriately respond to stimuli, dysregulation of plasticity can often result in disease. Epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) have been studied in the context of several diseases, including cancer biology [81]. Epigenetic mechanisms play a regulatory role in cellular plasticity; for example, miRNAs have been linked to EMT regulation [82]. In cancer, MET permits cells to dedifferentiate to a highly proliferative state and can result in tumor relapse with metastases after ending treatment [83]. Conversely, EMT increases invasion and dissemination of cells and decreases proliferation and thereby avoids cytotoxic chemotherapy agents [84]. The development of therapeutics that can inhibit cellular plasticity, such as inhibitors of transforming growth factor beta (TGF-β) [85] and c-Met, by blocking transition between cell states may increase the efficacy of specific agents relying on the target cell resembling a more primitive stem cell–like or differentiated phenotype. However, care must be taken to ensure such agents are administered to only the pathologic cells and not surrounding healthy cells to avoid off-target cytotoxic effects.

Epigenetic mechanisms lead to cellular heterogeneity and contribute toward development of treatment resistance. The methylation of histone H3K27 by EZH2 leads to transcriptional repression and has been linked to breast cancer aggressiveness [86]. Furthermore, H3K27me3 marks have been correlated with poorer outcomes in breast cancer during aromatase inhibitor therapy [87]. Resistance will spread from the initial cell that underwent epigenetic modifications to its daughter cells if the mechanism of epigenetic resistance has imprinting qualities. This presents a significant problem for the treatment of several chronic diseases such as diabetes, hepatitis, and cancer. The evolution of epigenetically adapting cells that develop resistance to treatment are similar to tumor cells with “driver” mutations that contribute to resistance [88]. In order to minimize potential resistance, combination therapy with other epigenetic modulators and/or existing therapeutics can be trialed.

**Epigenetic Therapies**

Characteristic epigenetic modifications predisposing to disease progression paves the way for the development of specific epigenetic therapies. Such interventions can be trialed in isolation or as adjuvants in combination with existing therapies. Because epigenetic alterations themselves can lead to adverse cell functionality via inappropriate plasticity transition, the specific epigenetic mechanisms underlying the pathological transition can be targeted to limit the progression of disease or even regress to a healthy phenotype. Such therapies would rely on an individual’s accurate epigenome data and would give rise to personalized epigenome therapy. Given the tight restrictions on clinical gene therapy, specifically the prohibition of
any treatment that can span generations, and the fact that epigenomic modifications can be heritable, similar restrictions should be considered for epigenetic modulation. Nonetheless, epigenomic modulation is an exciting avenue to explore the potential to limit disease in the research setting. Should this avenue of inquiry prove fruitful, then carefully designed clinical trials with ethical approval will be necessary to assess the suitability of epigenetic modification therapeutics.

Epigenetic modulating therapies have been trialed at the preclinical stage [89]. Hematologic malignancies have been targeted with DNA-hypomethylating agents such as 5-azacytidine (5-aza), which was the first drug approved by the Food and Drug Administration (FDA) for the treatment of myelodysplastic syndrome (MDS) in 2004 following two phase II and one phase III clinical trials. 5-aza has also been approved in Europe following the worldwide confirmatory Aza001 trial [90]. The pharmacokinetics of DNA-hypomethylating agent azacytidine have been studied in a phase I clinical trial comparing subcutaneous and oral administering of the drug in patients with MDS [91]. The orally administered drug was bioavailable and demonstrated biologic and clinical activity in patients with MDS. Therapeutics to epigenetically modify histones have also been trialed. HDAC inhibitors (HDACi) have been investigated in several clinical trials in hematologic and solid malignancies in isolation, but overall the efficacy was poor. Therefore, in several studies HDACi are used in combination with other anticancer treatments. Treatment of breast tumors with HDACi can improve responses to tamoxifen [92]. The combination of epigenetic modulators HDACi and DNA methyltransferase inhibitors like azacytidine for the treatment of hematologic malignancies is supported by the fact that abnormal recruitment of HDACs to nuclear protein complexes takes place in these malignancies [93]. This combination was shown to be effective, even in patients with relapsed disease who have previously received treatment with proteasome inhibitors [94]. HDACi therapies have been approved by the FDA; Virinostat (Zolinza) was the first HDACi to be approved by the FDA for the treatment of cutaneous lymphoma in 2006, and then Romidepsin was approved by the FDA in 2009 for the treatment of refractory cutaneous T-cell lymphoma [89].

Epigenetic drugs represent advancement in treatment modalities against certain cancers and have the potential to be developed for the treatment of other diseases with epigenetic components. The use of single agents and combination therapies both require treatment schedule optimization. Further research is also needed to adequately risk stratify patients to identify high-risk individuals that are most likely to benefit from epigenetic modulation therapy. The development of new epigenetic modulating therapies has the potential to improve existing clinical care and thus the overall health of the population.

CLINICAL APPLICATION OF EPIGENETIC BIOMARKERS—WHAT ARE THE PITFALLS?

Epigenetic biomarkers have the potential to significantly improve clinical practice by enabling risk stratification, providing an early diagnosis, and developing therapeutics. However, the use of epigenetic biomarkers has several potential pitfalls to be aware of, including multifactorial etiology of diseases, chemical influences, acquired drug resistance, and practical considerations. It is necessary to consider these possible limitations before epigenetic biomarkers are routinely adapted into clinical practice to prevent potential harms to patients.

Multifactorial Etiology of Diseases

Epigenetic biomarkers hold significant clinical potential for chronic diseases such as diabetes and cancer. These diseases are complex and have multifactorial etiologies. For example, there are many different well characterized types of cancer, and even each specific type has several different origins, including epigenetic influences. For instance, numerous different oncogenes can individually or synergistically transform a cell into a cancerous cell. The oncogenes expression level is in part regulated by epigenetic mechanisms such as promoter region methylation status, histone modifications, and miRNA expression profile. Therefore, aberrant epigenetic modifications can play a role in the development of disease. It is likely that the sum of local influences, epigenetic and others, determines gene expression status. Therefore, an epigenetic biomarker test that identifies one aspect of epigenetic regulation, like the methylation profile of a specific gene, can only be used as an indication of that specific gene’s expression level. Even if the gene promoter region is hypomethylated and transcription is active, downstream gene processing may be inhibited by miRNAs and therefore the gene may be expressed at a low level. Thus, the outcome of one epigenetic biomarker test may not reflect the global disease status. To overcome this potential issue, simultaneous epigenetic biomarker tests could be conducted and in conjunction with mRNA and protein expression tests. However, multiple testing requires more time and money and therefore may not be cost effective. Measuring global DNA methylation status may be a useful indicator for global gene expression, but it does not have the resolution to identify key genes. Therefore, measuring global methylation has potential to be used as an initial investigation, but further tests would be required. In the clinic, care should be taken when interpreting the results of epigenetic biomarkers not to overextrapolate the findings. Simultaneous epigenetic biomarker testing will help overcome this potential pitfall. Given the complexities of different levels of epigenetic modifications, it is important to understand the level of epigenetic variability that may be biologically significant and associated with risk.
Environmental Influence on Epigenetic Status

Epigenetic status is influenced by the environment [10,65]. There are several factors that synergistically alter an individual’s epigenetic status, including diet, toxins, drugs, aging, exercise, adiposity, and metabolism, reviewed by Ling and Groop [10]. It is therefore important to consider that these influences can alter the epigenetic status of a patient. At the same time, modifying the adjustable influences such as diet, exercise, and therapeutic drugs may offer a way of beneficially altering an epigenetic profile to lessen the burden of disease. Micronutrients like B₁₂ are necessary for appropriate nucleotide metabolism and vitamin B₁₂ insufficiency induces cholesterol biosynthesis by limiting s-adenosylmethionine and modulating the methylation of SREBF1 and LDLR genes [95]. Therefore, in a clinic, serum B₁₂ levels may need to be measured in addition to epigenetic testing. Emerging evidence indicates a close association between epigenetic modifications and metabolism and aging. DNA methylation profiles of several genes biologically relevant to the development of adiposity including lipase, hormone-sensitive (LIPE), that encodes hormone-sensitive lipase (HSL) in subcutaneous adipose tissue of adult men and women are associated with extent of adiposity [96]. Furthermore, enhanced biological aging in South Asian T2DM men with reduced telomere length tracked changes in lipids and BMI [97]. Therefore, in a clinical setting the age and metabolism of a patient should be considered when epigenetic analysis is conducted. Therapeutics can also influence epigenetic status. For example, DNA damage and repair can induce changes in DNA methylation [98]; therefore, cytotoxic chemotherapy can induce epigenetic changes. This has implications for the clinic because patients are often taking therapeutics and they may influence epigenetic status. Taken together, there are several environmental factors linked to epigenetic status that clinicians should consider when assessing patients’ epigenetic biomarker results.

Epigenetic Mechanisms of Acquired Drug Resistance

Epigenetic events that are somatically inherited through cell division have the potential to drive acquired drug resistance in cancer [99] and potentially other diseases where epigenetic changes in diseased cells provide a survival bias. Epigenetic modifications generate cellular heterogeneity [72] in which gene expression patterns can rapidly change to respond to the cellular niche, including adapting to therapeutic challenges, thereby leading to the development of acquired resistance. This phenomenon can complicate clinical practice where treatment decisions are made based on mutation biomarkers. It also strengthens the case for measuring epigenetic biomarkers to assist clinical decision making. Epigenetic states and acquired drug resistance have been reviewed by Brown et al., who suggest a strategy to prevent the emergence of resistance by targeting epigenetically “poised” states, where chromatin is in a bivalent form and can increase or decrease transcription of sets of genes in embryonic stem cells [99]. The authors argue that a DNA hypermethylation module associated with a stem cell gene signature in which the genes are epigenetically poised should be selected for during cancer development [100]. These points should be considered in the clinic when prescribing therapeutics that may interfere with epigenetic status to minimalize drug resistance.

Practical Considerations

Epigenetic biomarker testing is not currently in widespread use across clinical practice; therefore, it requires an implementation process to make ready the necessary equipment and training. This setup process is helped by the fact that several of the epigenetic biomarker analysis technologies are already in use in the clinical setting; for example, qRT-PCR can be adapted for miRNA screening. Nonetheless, cost-effective investigations for epigenetic biomarkers in the clinic should consider the long-term costs and benefits to allow for initial higher costs for hardware and training.

Epigenetic modifications are dynamic in their nature; therefore it is important to consider over what time period these changes take place. It is likely that different modes of epigenetic modification take different time periods to change. For example, histone modification dynamics rely on the availability of enzymes and substrates that may be available sooner than the time it takes to change an miRNA expression profile. Research is required to assess the dynamics of epigenetic modifications to assist clinical interpretation of epigenetic biomarker testing.

FUTURE PERSPECTIVES

Epigenetic biomarkers hold significant potential for use in the clinic to improve health prospects. To assist the implementation of clinical epigenetic biomarkers, several courses of action should be followed. Further research is needed at every stage of epigenetic biomarker understanding. Although DNA methylation, histone modifications, and miRNAs have been identified, there is much to learn about how these epigenetic processes are regulated and the crosslinks between mechanisms [14]. Furthermore, there are likely to be additional undiscovered epigenetic processes that may hold potential for clinical use.
Although technology exists to measure epigenetic biomarkers, there is scope to improve it. The clinical use of epigenetic biomarkers will greatly benefit if the current technology is modified to deliver quicker results. This is achievable through faster and more simplified sample processing and high-throughput analysis procedures. Technologies to generate kinetic models of posttranscriptional regulation by miRNAs are needed to advance the understanding of disease progression. Next-generation technology for profiling miRNA has been developed, but it is currently not cost effective. Therefore, strategies to improve cost-effectiveness of technology should be adopted.

To build on existing knowledge of characteristic epigenomic changes in specific diseases, more detailed studies of the progression of disease should be conducted. A prioritization should be placed on characterization of epigenetic changes during the initial phases of abnormal physiology leading to disease. This will provide a reference epigenetic biomarker profile with which to compare the epigenomic profile of a patient. Because epigenetic changes occur prior to alterations in gene expression potentially leading to a disease state, epigenetic changes themselves may provide an early marker of potential alterations in gene expression leading to disease. Epigenome Wide Association Studies (EWAS) should be conducted by determining profiles of methylation, histone modifications, and miRNA in samples from healthy subjects and patients with disease to identify disease-associated epigenetic marks. Individual miRNAs should be further investigated to assess their role on target genes and clusters of genes.

Panels of several key epigenetic modifications can be generated for certain diseases. Having several different epigenetic modifications will reduce the false-positive results compared to one epigenetic biomarker. These panels will act as a tool to assist clinical practice for diagnosis, risk stratification, and chemoprevention. Epigenetic biomarkers have the potential to be used to assist earlier clinical diagnosis. Subsequently, this will allow earlier intervention and likely to improved health outcomes. Risk stratification will enable high-risk patients to be identified and allow the option of chemoprevention.

Further research should be conducted to explore the links between epigenetics and environmental factors such as aging and metabolism. Metabolomics, an emerging field that provides novel information about biological perturbations based on changes in multiple small-molecule metabolites, has the potential to help decipher the delicate interactions between metabolism and the genome. Epidemiological epigenetic biomarker studies may provide valuable insight into the variation in biomarkers across populations.

Epigenetic therapeutics have shown promise in early clinical trials. More clinical trials with multivariate analyses with large numbers of patients and long-term follow-up are needed to establish the epigenome altering effects in vivo. Research should focus on pharmacological agents that can target specific genes and also a new class of epigenetic modifiers that can mediate demethylation.

CONCLUSION

Much advancement has been made in the understanding of phenotype through regulation of the genome by epigenetic mechanisms. Advancing technologies have enabled the study of epigenetics to help reveal how changes in gene expression are regulated in physiological and pathophysiological conditions. These findings have significant clinical potential. Epigenetic changes occur very early in the transition to disease state and therefore can be used as biomarkers to make earlier diagnoses, carry out risk stratification and assist with the option of chemoprevention. Furthermore, epigenetic modifications have several therapeutic implications; they can be used to assess the effectiveness of a therapeutic intervention and they can be directly targeted with epigenomic modifying agents. Further research is needed to better understand how epigenomic modifications are regulated and how they interact with the environment. This information will assist the development of tools for clinical practice. Complete epigenetic profiles through disease progression will help clinical prognosis and patient management. Further research is needed to explore the potential of epigenetic therapeutics. The ability to modify gene regulation through epigenetic therapy may be able to rectify aberrant epigenetic profiles in diseased cells to stop progression of the disease thereby rescuing a healthy phenotype.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACF</td>
<td>Aberrant crypt foci</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacillus Calmette–Guerin</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Breast cancer 1, early onset</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CIMP</td>
<td>CpG island methylator phenotype</td>
</tr>
<tr>
<td>CpG</td>
<td>Cytosine–guanine</td>
</tr>
<tr>
<td>CRISPR</td>
<td>Clustered regularly interspaced short palindromic repeats</td>
</tr>
<tr>
<td>cRNA</td>
<td>Complimentary ribonucleic acid</td>
</tr>
</tbody>
</table>
DNA  
Deoxyribonucleic acid

DNMT (1, 3a, 3b)  
DNA methyltransferase (type 1, 3a, 3b)

dsDNA  
Double-stranded deoxyribonucleic acid

EBVaGC  
Epstein–Barr virus–associated gastric carcinoma

EMT  
Epithelial to mesenchymal transition

GWAS  
Genomewide association studies

H (2A, 2B, 3, 4)  
Histone (type 2A, 2B, 3, 4)

HAT  
Histone acetyltransferase

HbA1c  
Glycated hemoglobin

HDAC  
Histone deacetylase

HDM  
Histone demethylase

hMLH1  
Human mutL homolog 1

HMT  
Histone methyltransferase

HPCE  
High-performance capillary electrophoresis

HSL  
Hormone-sensitive lipase (protein)

LIPE  
Lipase, hormone sensitive (gene)

MDS  
Myelodysplastic syndromes

MET  
Mesenchymal to epithelial transition

MGMT  
O6-methylguanine–DNA methyltransferase

miRNA  
micro-ribonucleic acid

ncRNA  
Noncoding ribonucleic acid

NHS  
National Health Service

NICE  
National Institute for Health and Care Excellence

PCR  
Polymerase chain reaction

PSA  
Prostate-specific antigen

qRT-PCR  
Quantitative real-time polymerase chain reaction

RNA  
Ribonucleic acid

T2DM  
Type 2 diabetes mellitus

TB  
Tuberculosis

TGF-β  
Transforming growth factor beta

UV/Vis  
Ultraviolet to visible

WHO  
World Health Organization

REFERENCES


Epigenetic Biomarkers of Disease  Chapter | 10  175


