Understanding and Treating Epigenetic Drivers of Cancer

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ABSTRACT

Epigenetic modifications, primarily DNA methylation and histone deacetylation, are known to lead to certain cancers due to their effects on tumour suppressor genes and oncogenes. However, there have been limitations on how our understanding of these modifications can provide effective treatments and preventative measures for patients. This article summarises our current understanding of cancers affected by epigenetics, epigenetic therapies in the form of inhibitors, and the use of epigenetic factors as prognostic and predictive biomarkers for patients. Within the development of cancers caused by epigenetics, this article examines differences between sporadic and hereditary cases for colorectal, breast, and ovarian cancer. In terms of treatments, this article lists some of the most well-known DNA methylation and histone deacetylase inhibitors currently under clinical investigation and which cancers they have the potential to treat. Finally, this article explores the possible biomarkers for epigenetic cancers, considering cell-free DNA and microRNA. This article summarises that future studies should explore a variety of factors regarding the causes, treatments, and identification of epigenetic cancers to maximise patient care.

Introduction

Epigenetic changes can interfere with transcription of specific genes, causing different genes to be expressed. This begins as DNA is labelled by epigenetic marks such as methyl groups, which inhibit gene expression, and acetyl groups, which promote gene expression. Demethylation and acetylation can also occur which remove methyl and acetyl groups from DNA bases, respectively. These marks commonly attach to histones, where histone methylation leads to DNA being more tightly wrapped around histones and being inaccessible to transcription factors. Histone acetylation leads to the opposite, with DNA being less tightly wrapped around histones allowing for greater access to transcription factors.

Several factors affect which marks are present ranging from diet, chemical exposure, and radiation. A popular example of external factors influencing gene expression is seen through Agouti mice. The usual phenotype for Agouti mice involves brown fur and an average weight, which is normally achieved when pregnant mice consume food high in methyl groups. These foods can decrease expression of the agouti gene in the mice’s offspring. However, when this food is not consumed, the Agouti gene is actively transcribed, causing mice to be obese and yellow. Figure 1.

Through this well-known example, it is noticeable how heavily epigenetic marks can affect the phenotype of an organism. However, in some cases, the irregular presence/absence of marks can lead to much more serious and devastating health outcomes. Many recent findings have shown the effects of epigenetic marks on cancers, through marks attaching to oncogenes and tumour suppressor genes. This irregular epigenetic mark placement can lead to a variety of cancers such as breast and colorectal cancer. Furthermore, the inheritance of these marks may also be a possibility, leading to several generations being affected by epigenetic cancers. In the following sections, this article explores some common cases of epigenetic cancers and the potential of inhibitors as a treatment.
Figure 1. A diagram depicting the effects of methylation in the phenotypes of Agouti mice. The diagram shows the effect of agouti mice consuming methyl-rich foods, which cause methyl groups to bind to the promoter region of the agouti gene, preventing its transcription. Hence, the mice have brown fur and are of an average weight. However, when methyl-rich foods are not consumed, the agouti gene is activated, and its effects are enabled with mice having orange fur and obesity.

Lynch Syndrome

Lynch syndrome is a particularly prominent case of cancers caused by epimutations\(^3\). It is the most common hereditary form of colorectal cancer, with an estimated 134,490 cases and 49,190 deaths in 2016 in the US\(^4\). The disease is caused by a germline mutation in the mismatch repair genes, such as MLH1, MSH2 and MSH6\(^3\).

Lynch syndrome is an example of the variety of epigenetic-related cancers thought to be hereditary\(^5\). There are also cases of epimutations through promoter methylation and transcriptional silencing of a single allele (when there are no sequence mutations) which is thought to lead to early-onset lynch syndrome\(^5\). Exploring early-onset cancer further can be a gateway into preventing cancers through inhibitor therapies at a stage where they can be most effectively treated.

Inheritance

As for its generational link, Lynch syndrome has a constitutional epimutation linked to an MLH1 haplotype with two single-nucleotide variants in separate Caucasian families in Australia\(^6\). This findings indicate that
there is a common European ancestral haplotype that has epigenetic variants which lead to Lynch syndrome, which supports the basis for transgenerational epigenetic mark inheritance [6]. This trans-generational link may be particularly useful to identify preventative and predictive solutions using epigenetic technologies for relatives of patients who are at an elevated risk of developing the disease.

However, the process of carrying epimutations has a few restrictions. Hitchins et al [7] examined twenty-four early-onset patients with either colorectal cancer or endometrial cancer at a genetic level. Two women with hemiallelic methylation of MLH1 in all their somatic cells were identified, but when the sperm of one of their sons was analysed to identify the likelihood of epimutation inheritance to his offspring, no trace of methylation was found. RNA analysis found that there was a reactivation of MLH1 expression, indicating that spermatogenesis reversed the MLH1 epimutation [8]. This suggests that the most effective manner to identify potential patients may be through a trans-generational maternal link. Further research into the inheritance of epigenetic cancers can allow for high-risk cases to be more accurately considered and prevented at a faster rate.

Sporadic Cases

Although many cases of lynch syndrome are thought to be germline, there have been cases of sporadic lynch syndrome. For instance, a hypermethylation of hMLH1 was found in normal blood DNA of a patient whose parents did not have the same mutation [9]. For early onset sporadic colorectal cancer, complete methylation of the hMLH1 promoter region in peripheral blood lymphocytes is often identified. While epigenetic therapies have been thought to aid this process, using a combination of DNMT and HDAC inhibitors cannot remodel actual chromosomes, hence has no effect on the expression of stable genes [9]. Thus, whilst generational cases of cancers such as lynch syndrome do provide some basis for an implementing accurate and effective preventative care, it is not a guarantee that this care will be fully sufficient, and a further understanding on the causes of sporadic cases must be established.

Breast & Ovarian Cancer

Other cancers significantly related to epigenetic mutations are breast and ovarian cancers. Familial and early onset cases of breast cancer are thought to be due to a hypermethylation of the BRCA1 and BRCA2 gene [10]. CpG islands, the areas of DNA which contain cytosine and guanine, in the regulatory regions of genes are unmethylated. However, the process of methylation can lead to condensed chromatin structure, which may contribute to breast cancer [10]. An epigenetic screening for ovarian and breast cancer may allow better strategies for testing susceptibility, hence, have high beneficial clinical outcomes.

Mutations of the ARID1A gene have been related to ovarian cancers, however, the effects of these mutations can be reduced through therapies [11]. Studies such as Bitler et al [12] have shown that inhibiting EZH2 methyltransferase reduced ARID1A-mutated tumours of ovarian cancer in mice, which indicates the positive impact of methylation inhibiting therapies for cancers. However, for some patients, there is still an issue of an intense immune response towards the drugs targeting transcription factors [12]. Thus, further research must be conducted to analyse how therapies for breast and ovarian cancers can be practically implemented without a risk of a side effect or adverse events.

Twin Studies

Many epigenetic conditions are often assessed for variation between external and biological factors. A common method to assess epigenetic variation are twin studies, which were used to analyse the development of breast cancer. Twin studies can be conducted through genetic analysis to identify susceptibility loci that could be
modified by external factors. In twin studies where a MZ (monozygotic) pair was examined, an increase of methylation for the BRCA1 gene was identified for the affected twin\textsuperscript{13}. This study is supported by the finding that the hypermethylation of the BRCA1 promoter region has been commonly identified in sporadic non-familial breast cancer\textsuperscript{13}. Thus, similarly to Lynch syndrome, breast cancer has found to display both sporadic and hereditary cases which must be researched further to effectively implement predictive measures for high-risk individuals.

**Inhibitors (Acetylation and Methylation)**

**DNMT-Is**

Common epigenetic therapies to reduce the risk of cancers include inhibitors, which can alter the effects of methylation and acetylation. DNA methylation inhibitors (DNMT-Is) are split into two classes of nucleoside analogues and non-nucleoside analogues\textsuperscript{14} (Table 1). They stop the methylation of useful genes (such as tumour suppressor genes), inhibiting their abnormal lack of expression which leads to cancer and tumour formation\textsuperscript{14}. However, whilst DNA methylation inhibitors do have practical application, they often lack specificity and cause genome-wide hypomethylation which can activate genes which are rightly silenced. These issues can be overcome by chemically synthesising drugs as small molecules to have more target specificity\textsuperscript{14}. Overall, DNMT-Is have a strong potential in treating types of cancers with a methylation epimutation and have multiple clinical benefits.

**HDAC-Is**

Conversely, histone deacetylase inhibitors (HDACIs) prevent deacetylation. HDACIs are categorized into four classes of short chain fatty acids, hydroxamic acids, cyclic peptides and synthetic benzamides\textsuperscript{15} (Table 2). They promote the acetylation of histones which loosens the structure of the chromatin tail, hence, transcription factors can access the DNA and enhance gene transcription. They can reactivate expression of hypermethylated or silenced tumour suppressor genes, preventing cancer and tumour formation if tumour suppressor genes are reactivated\textsuperscript{15} (Figure 2). However, there are issues of HDACI, particularly due to a lack of knowledge about dosage and resistance. With additional research, a better understanding about the use of HDAC-Is as a treatment to deacetylation epimutations can be developed\textsuperscript{15}. 
Figure 2. A diagram depicting the effects of HDAC-Is on cancers caused by HDACs. The diagram depicts the shortening of the chromatin structure when HDAC enzymes attach to histones, which leads to cancerous activity due to the inaccessibility of transcription factors. It then displays how HDAC-Is allow better access to transcription factors as acetylation, leading to anti-cancer activity.

HDACIs have a clinical use for cervical cancer, where HPV triggers a host defence system upon the insertion of the viral gene such as methylation machinery activation\cite{16}. This could have a basis for the carcinogenic process which follows, such as global DNA hypomethylation, hypermethylation of key tumour suppressor genes, and histone modifications. Studies have suggested that HDAC-Is such as 5-aza-2-deoxycytidine plus cisplatin can reactivate expression of hypermethylated or silenced tumour suppressor genes\cite{16}, which may potentially lead to greater tumour suppression and an effective treatment for cervical cancers. Research such as this is vital to deduce the clinical effects of inhibitors on types of cancers, and similar research must be conducted using specific HDAC-Is to identify treatments for other cancers.

Other tested HDACIs include MS27, which was studied on various cell lines consisting of malignant ascites\cite{17}. MS27 was found to increase apoptosis of ascites cells, which may be able to act as a treatment. The inhibitor could also downregulate proteins which are related to the progression of the cell cycle, suggesting it could stunt rapid division\cite{17}. However, further studies should look at a more clinically relevant approach to utilising MS27, as merely exploring its effects on ascites could suggest that the inhibitor may not be effective for successful recovery at earlier stages. Although, the analysis of the effects of HDACIs in both ascites and in clinical cervical cancer demonstrates the versatility of these inhibitors.

Inhibitor Tables (Tables 1 & 2)

Below are two compiled tables of different types of inhibitors: one for DNA methylation inhibitors (Table 1) and the other for histone deacetylase inhibitors (Table 2). DNA methylation inhibitors are categorised into nucleoside analogues and non-nucleoside analogues dependent on their structures and where they bind to. The histone deacetylase inhibitors listed on the table are split into short-chain fatty acids and hydroxamic acids.
These are also determined by the structure of the inhibitor and their functional groups. The table also includes the types of cancers the inhibitor is likely to be used against (some effective in animal models) and the stage of clinical trials the inhibitor is within.

**Table 1. DNA Methylation Inhibitors**

<table>
<thead>
<tr>
<th>Names</th>
<th>Type/structure</th>
<th>Cancer</th>
<th>Clinical Trials</th>
<th>Citations</th>
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<tbody>
<tr>
<td>Names</td>
<td>Type/structure</td>
<td>Cancers</td>
<td>Clinical Trials</td>
<td>Citations</td>
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Table 2. Histone Deacetylase Inhibitors
<table>
<thead>
<tr>
<th>Compound</th>
<th>Type</th>
<th>Indications</th>
<th>Stage</th>
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<tbody>
<tr>
<td>Pyroxamide</td>
<td>Hydroxamic acids</td>
<td>Haematological malignancies</td>
<td>Preclinical</td>
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<tr>
<td>Scriptaid</td>
<td>Hydroxamic acids</td>
<td>Multiple myeloma</td>
<td>Preclinical</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Suberoylanilide hydroxamic acid (SAHA)</td>
<td>Hydroxamic acids</td>
<td>Haematological malignancies, solid tumours</td>
<td>Phase I, II</td>
</tr>
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<tr>
<td>Trichostatin A (TSA)</td>
<td>Hydroxamic acids</td>
<td>PC3 prostate cancer</td>
<td>Preclinical</td>
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<td>LBH589</td>
<td>Hydroxamic acids</td>
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<tr>
<td>MS275</td>
<td>Hydroxamic acids: pan-HDAC inhibitors SAHA</td>
<td>Cervical cancer</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

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Biomarkers

Early Biomarkers – Cancer Risk

Epigenetic changes in cancer present an opportunity not only for treatment through epigenetic therapies, but also for preventative measures such as biomarkers. Particularly, consistency in abnormal DNA methylation for certain cancers has allowed their use as diagnostic methods of malignancies. For instance, the epigenetic silencing of the MLH1 gene can be used as a differential diagnosis of non-heritable CRC and Lynch syndrome\[31\]. The process of differential diagnosis involves a 2-level screening test with an analysis of a loss of DNA mismatch repair (MMR) expression and a positive test for microsatellite instability (MSI). If this is found, then analysis of MLH1, MSH2, MSH6, PMS2, or EPCAM is conducted\[31\]. This constitutional epimutation testing is used to confirm Lynch syndrome. Thus, a patient can be appropriately treated through obtaining the most accurate diagnosis, ensuring that treatment is quick and does not increase the severity of the cancer.

Biomarkers After Diagnosis

*Predicting Cancer Severity/Metastasis*

In addition to acquiring a cancer diagnosis at an early stage, biomarkers can also be used to predict the severity of the cancer for a patient. When DNA is pre-tested with bisulphate, DNA methylation can be analysed using high throughput sequencing\[32\]. A study which used this method of DNA analysis identified the DMR (differentially methylated region) status in early-stage colorectal cancer which could be used to predict metastasis and found that the majority were hypermethylated in the unfavourable prognosis group\[32\]. This may suggest that the same testing on existing cancer patients could be conducted to search for a similar hypermethylation. Predicting metastasis could be a major support for the treatment plan for the patient going forward, ensuring that they receive the best care for the stage they are within.
**Predicting Treatment Responses**

Furthermore, biomarkers could be used to predict the responses patients may have to treatments (Figure 3). This is especially key to prevent any adverse side effects which an unsuitable treatment may impose on a patient. Since cancer patients are already in a vulnerable state, further complications may drastically worsen their health. Thus, a prediction for treatment response is essential to ensure overall patient safety. A way in which prognostic marker detection can take place is through the molecular profiling of circulating cell-free DNA (cfDNA). In addition to being highly beneficial in the initial treatment of cancer, it is also minimally invasive and highly precise \(^{[33]}\). For instance, the hypermethylation of ESRI and CYP1B1 is associated with improved clinical outcome from endocrine therapy \(^{[33]}\). In addition to treatment response, distinct types of cfDNA may yield different uses as biomarkers, making it a highly versatile and resourceful method of analysis which must be used much more in future studies and treatment plans.

**MicroRNAs/cfDNA**

Further applications of analysing cfDNA in oncology include tumour profiling, therapy response monitoring and recurrence detection. Many of these can be found through analysing somatic mutations in ctDNA (circulating tumour DNA), which come from tumours and cancer cells, after cell death \(^{[34]}\) (Figure 3). However, there are some difficulties in extracting the epigenetic changes of cfDNA as they will have to be obtained through indirect methods, and the complexity of cfDNA makes tracing the cell origins of the epigenetic marks difficult \(^{[34]}\). Thus, although an analysis of cfDNA such as ctDNA can provide immense information on a patient throughout their cancer treatment, much of this information may be unsuitable without a method to identify the specificities of the cfDNA itself, which requires much more research and analysis to use the most effectively.

Other than ctDNA, cf-mtDNA (cell-free mitochondrial DNA), which is usually the result of a disruption to the mitochondrial life cycle, can also be used as a biomarker for cancer patients. mtDNA is thought to have prognostic benefits, with studies showing higher cf-mtDNA levels in head and neck cancer and ovarian cancer patients compared to controls \(^{[35]}\). An increase in cf-mtDNA levels has also been related to the progression of cancer, particularly in lymph node metastasis, which suggests its predictive uses for cancer survival \(^{[35]}\). Overall, cf-mtDNA has both the predictive and prognostic benefits needed for it to thrive as a highly effective and practical biomarker (Figure 3).

In addition, ecDNA (extra-chromosomal DNA) is another form of cfDNA which has beneficial effects as a biomarker. As a prognostic biomarker, the length of ecDNA can indicate cancer, with lung cancer patients having longer ecDNA compared to controls \(^{[36]}\). Furthermore, ecDNA can also be biomarkers for early diagnosis for lung adenocarcinomas \(^{[36]}\). In addition to acting as an early biomarker, the amplification of certain ecDNA has been correlated with drug resistance (Figure 3). Specifically, MET ecDNA amplification has been correlated to a resistance to ROS1 tyrosine kinase inhibitors \(^{[36]}\). Through its use as both a predictive and prognostic biomarker, ecDNA is another versatile form of cfDNA which may be used to improve the rate and efficacy of care which patients receive. As aforementioned, its particular use for identifying patients’ resistance towards inhibitors highly improves the quality of care which patients receive through the prevention of developing life-threatening side effects.

As we identify a greater potential of cfDNA as a biomarker for cancer epigenetics, it is essential to evaluate how to improve cfDNA analysis. Currently, cfDNA is commonly processed with bisulphate or the conversion of unmodified cytosines into uracil \(^{[37]}\). However, these methods commonly have low yields, which led to the development of a single-molecule sequencing approach. This may provide a higher yield as sequencing libraries can be generated from nanogram amounts of cfDNA per sample \(^{[37]}\). While sequencing cfDNA of twenty patients with colorectal cancer using this method, it was found that the variation of genome-wide methylation in cancer patients was 7% compared to less than 2% for healthy participants \(^{[37]}\). Therefore, by acquiring these accurate and functional results using a single-molecule sequencing approach, we can determine that the
process of cfDNA analysis is consistently improving to combat the challenges which previous methods faced. Overall, a greater call for research in cfDNA analysis can allow for a higher precision in the care of patients.

![Figure 3](image)

**Figure 3.** A diagram depicting the different types of cfDNA with a description of their role as biomarkers. The diagram shows the origin of cfDNA in the bloodstream from necrosis and apoptosis. It also displays the uses of distinct types of cfDNA. cfDNA as a whole and mtDNA are shown to be used as a prognostic and predictive biomarker, ctDNA with uses for tumour profiling and recurrence detecting, and ecDNA as an indicator for drug resistance.

Other than cfDNA, microRNAs (miRNA) can also be used as cancer biomarkers. miRNA are small molecules of RNA which are often not considered in genetic analysis, but still affect gene expression. The pathway for miRNA biogenesis involves primary miRNA being processed by the Drosha enzyme and the DGCR8 protein into pre-miRNAs. These pre-miRNAs are transferred into the cytoplasm, and then matured into single-stranded miRNAs by the Dicer proteins. miRNA can act on tumour suppressor genes and oncogenes, and an aberrant miRNA expression has been linked to cancer development (Figure 4).

miRNAs have shown to have significant differences between cancer patients and controls when used as biomarkers. For instance, a study found that the analysis of microRNA methylation levels has a use as diagnostic biomarkers for ulcerative colitis (UC)-associated colorectal cancer. DNA methylation silencing of miR-124 was also found to be a marker for improved detection of cervical cancer. Furthermore, the methods of miRNAs collection from patients are also beneficial, through the common use of liquid biopsies. Since surgical biopsies have excessive costs and risks, liquid biopsies are a beneficial alternative that provide diagnostic information about a range of tumours. Overall, though miRNAs are often overlooked in many gene analysis studies, they may have a crucial role in cancer epigenetics which must be researched more effectively. Their use as a diagnostic biomarker may massively reduce the progression of certain cancers by allowing treatments to be administered as early as possible.
**Figure 4.** A diagram depicting the biogenesis of miRNA and the methods by which it may cause cancer. This diagram displays primary miRNA being processed by the Drosha enzyme and the DGCR8 protein, and made into pre-miRNA. The pre-miRNA is shown to be transferred into the cytoplasm and matured into miRNA by dicer proteins. The cancerous effects of miRNA are displayed by miRNA either binding to mRNA, leading to degraded mRNA, or through miRNA binding to the promoter region of oncogenes, thus, transcribing them.

**Conclusion**

Overall, this article has considered how epimutations can lead to different cancers and how our scientific understanding of the causes of epigenetically linked cancer can be used to cater treatments. In the future, more research must be conducted considering a variety of different areas. In particular, understanding the causes of early-onset cancer (specifically in relation to lynch syndrome) are crucial to provide the most accurate epigenetic therapies which can prevent significant progression of cancer. Furthermore, greater genetic analysis must be implemented to identify high-risk patients from hereditary cases in order to provide preventative care. For inhibitors, research on both HDAC-Is and DNMT-Is must be conducted to establish any adverse effects or drug resistance which patients may have (which could be explored through use of biomarkers). In addition to treatment response, predictions of metastasis are vital to understand the progression of the patient and effectively adapt their care. For biomarkers, cfDNA must be investigated further, seen as new methods of nanogram analysis have yielded impressive results. The role of miRNA as biomarkers must also be considered, with many studies overlooking their significant correlation with cancer. Whilst we obtain a greater level of understanding of epigenetic cancers, the scientific community must assess these findings more in order to provide the most effective and reliable care for patients.
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References


