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Cancer epigenetics in solid organ tumours: A primer for surgical oncologists

Thomas M. Drake [a], Kjetil Søreide [a, b, c, d, *]

[a] Department of Clinical Surgery, Royal Infirmary of Edinburgh, University of Edinburgh, Edinburgh, UK
[b] Gastrointestinal Translational Research Unit, Laboratory for Molecular Biology, Stavanger University Hospital, Stavanger, Norway
[c] Department of Clinical Medicine, University of Bergen, Bergen, Norway
[d] Department of Gastrointestinal Surgery, Stavanger University Hospital, Stavanger, Norway

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ABSTRACT

Cancer is initiated through both genetic and epigenetic alterations. The end-effect of such changes to the DNA machinery is a set of uncontrolled mechanisms of cell division, invasion and, eventually, metastasis. Epigenetic changes are now increasingly appreciated as an essential driver to the cancer phenotype. The epigenetic regulation of cancer is complex and not yet fully understood, but application of epigenetics to clinical practice and in cancer research has the potential to improve cancer care. Epigenetics changes do not cause changes in the DNA base-pairs (and, hence, does not alter the genetic code per se) but rather occur through methylation of DNA, by histone modifications, and, through changes to chromatin structure to alter genetic expression. Epigenetic regulators are characterized as writers, readers or erasers by their mechanisms of action. The human epigenome is influenced from cradle to grave, with internal and external life-time exposure influencing the epigenetic marks that may act as modifiers or drivers of carcinogenesis. Preventive and public health strategies may follow from better understanding of the life-time influence of the epigenome. Epigenetics may be used to define risk, to investigate mechanisms of carcinogenesis, to identify biomarkers, and to identify novel therapeutic options. Epigenetic alterations are found across many solid cancers and are increasingly making clinical impact to cancer management. Novel epigenetic drugs may be used for a more tailored and specific response to treatment of cancers. We present a primer on epigenetics for surgical oncologists with examples from colorectal cancer, breast cancer, pancreatic cancer and hepatocellular carcinoma.

Introduction

Understanding the processes driving the initiation, progression and behaviour of solid tumours at the cellular level is a core part of the curriculum for surgical oncologists [1]. As research and knowledge surrounding cancer biology rapidly develops, previous knowledge becomes outdated and requires updating. Thus, experienced and young surgeons alike require information to appreciate the molecular biology of solid visceral tumours in order to understand and deliver high quality cancer care.

Epigenetics - the study of molecular changes to the DNA that do not arise from alterations in the DNA proper - is of utmost interest and importance for research into various diseases processes, including cancer [2,3]. The epigenetic information in human cells is influenced through various levels and continuously during a life-time (Fig. 1) of exposures to both health promoting and potential toxic factors [4]. Alterations may occur through genomic changes, as an effect of internal or external exposures and through random effects. Thus, epigenetics stands at the interface of the genome, human development, and environmental exposure [5–7]. Consequently, epigenetics may be influenced at several levels and at various timepoints in humans to initiate or modify risk of disease such as cancer. Indeed, cancer itself may alter the epigenetic code through exposures and selection pressure during the various phases of malignant transformation.

Epigenetic alterations occur in concert with other genetic changes (Fig. 2) to influence the genomics, transcriptomics and proteomics that drive the cancer phenotype [3,5,8,9]. Epigenetic

* Corresponding author. Department of Gastrointestinal Surgery, Stavanger University Hospital, POB 8100, N-4068, Stavanger, Norway.
E-mail address: ksoreide@mac.com (K. Søreide).

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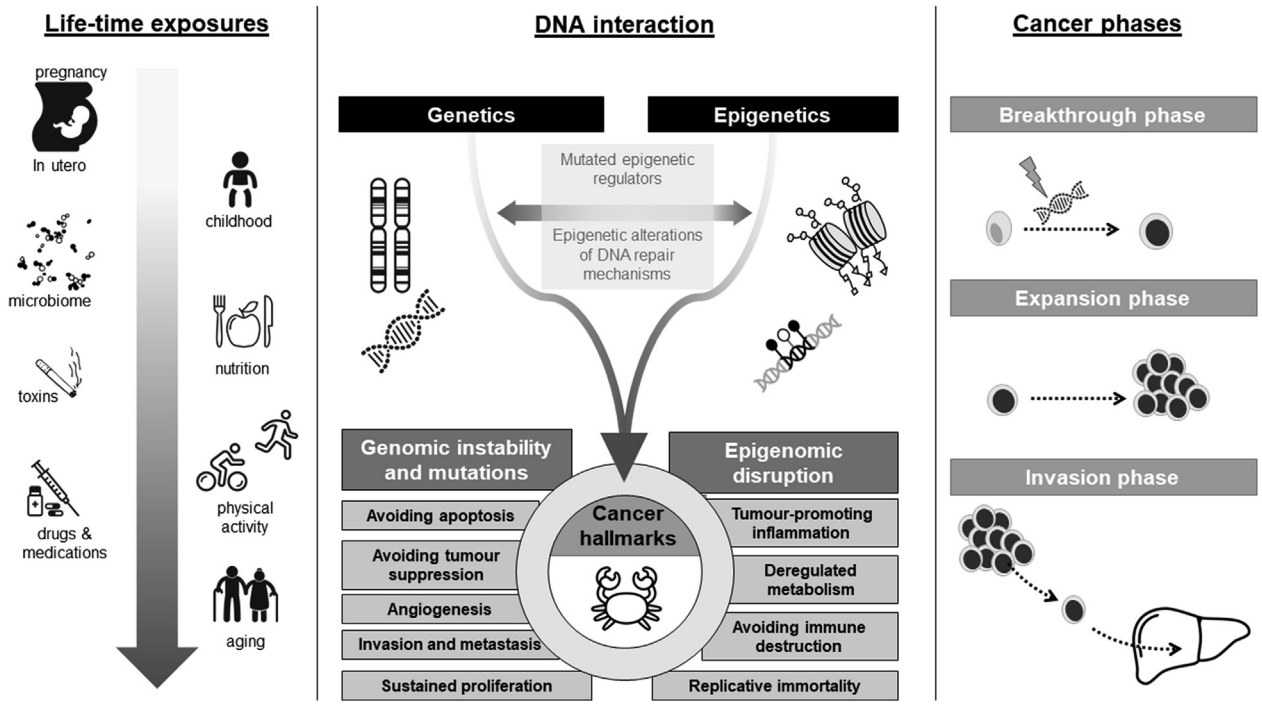

Fig. 1. Epigenetic influence through a lifetime of exposure, DNA interactions and cancer development.
Epigenetic alterations occur throughout a human life, from in utero exposure (left panel) until ageing processes start. The DNA interaction (middle panel) that occurs are mutually interacting and influencing the cancer hallmarks for cancer risk and progression, for which current research seeks to explore the exact mechanisms a contribution throughout the phases (right panel) of cancer development. The illustration is generic and unique patterns of exposure, risk and influence may occur to each specific cancer type.

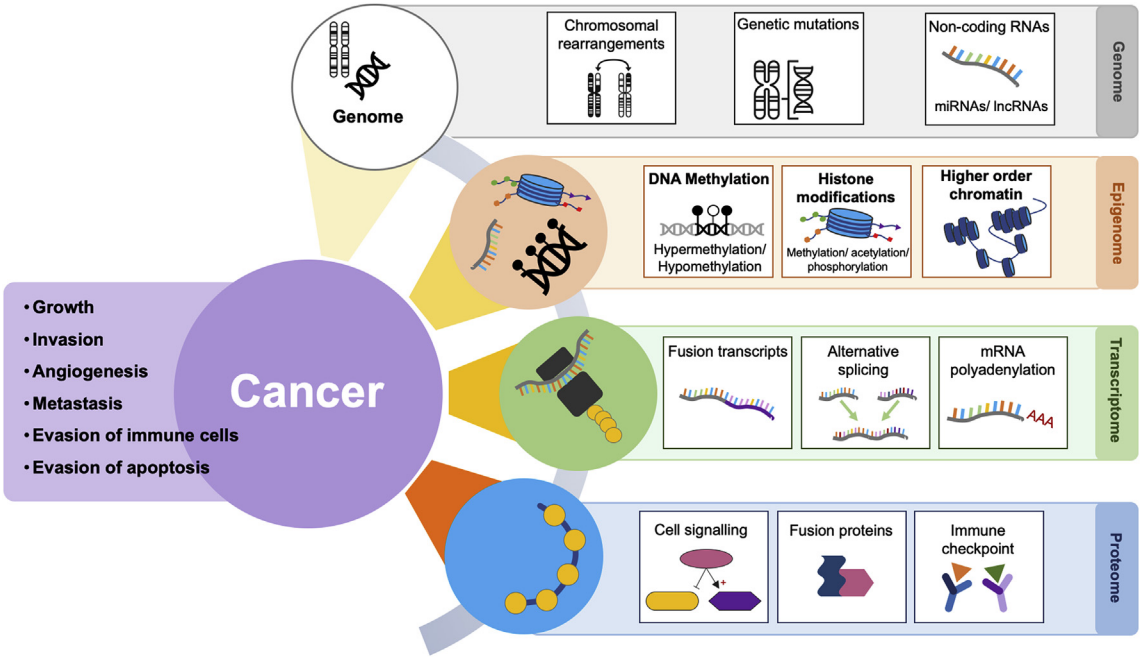

Fig. 2. Overview of 'omics' in cancer regulation.
Epigenetics broadly occur as DNA methylation, histone modifications, and higher-order chromatin regulation. Epigenetics are influenced by the genome, transcriptome and proteome and thus is an important part of the complexity of cancer development.

changes involve DNA methylation, histone modifiers and readers, chromatin remodellers, microRNAs, and other components of chromatin [10]. Cancer genetics and epigenetics are linked in generating the malignant phenotype; i.e. epigenetic changes can cause mutations in genes (Fig. 1), and, conversely, mutations are frequently observed in genes that modify the epigenome [9]. Epigenetics is increasingly becoming incorporated into routine clinical practice, for example in glioblastoma multiforme, where O-6-Methylguanine-DNA Methyltransferase (MGMT) methylation status is used to guide Temozolomide therapy.

Epigenetics has been associated with cancer since the 1980s, but only more recently has the potential for clinical use in form of improved tumour classification, as epigenetic biomarkers or as novel targets of therapy [11,12]. In this review we will explore some of the key areas where cancer epigenetics play a current role in solid visceral tumours in general and for selected solid organ cancers specifically. As frequently investigated cancer models and public health burden, we chose to focus on colorectal and breast cancer. Also, we chose hard to treat cancers with a current overall poor prognosis, such as pancreatic cancer and hepatocellular carcinoma. All the cancer types discussed herein are frequently represented in the scope of surgical oncology practice and should thus be of relevance to the practicing clinician. Current updated reviews from the past 3–5 years period are selected to allow the interested reader to seek further in-depth knowledge to specific topics.

Methods

This narrative review was based on a literature search of the PubMed database covering the last decade, with a strong emphasis on papers and studies published over the last five-year period (up to January 10th, 2019). Authorative reviews were chosen to allow the interested reader to search further indepth knowledge and studies relted to the selected cancers were chosen as examples. We acknowledge that the search is not exhaustive and apologize to those authors whose work could not be cited.

The mechanisms of epigenetic regulation

Cancer has long been viewed simply as a genetic disease. Changes to the human genetic code – such as mutations, copy number alteration, insertions, deletions, or recombinations – are particularly well suited to induce persistent phenotypic changes in cancer. However, sporadic genetic events occur at a low frequency and are thus not a very efficient way of causing malignant transformation [13]. Some cancer cells overcome this bottleneck by acquiring DNA repair defects, thus boosting the mutation rate - such as seen in mismatch repair deficient tumours that lead to high occurrence of microsatellite instability (MSI) throughout the genome [14,15]. Mechanisms of epigenetic control offer an alternative path to acquiring stable oncogenic traits [9,16].

Epigenetic states are flexible, persist through multiple cell divisions and, influence the cellular phenotype. Broadly, epigenetics occurs through 3 different forms as has recently been described in detail elsewhere [2,3]; through methylation of DNA; post-translational modification of nucleosomal histones and, lastly, through organization of higher-order chromatin structure (Fig. 2). Closely related mechanisms are increasingly being tied to epigenetic regulation and control of the 'epigenetic landscape', such as non-coding RNA [17,18] (as either small or long non-coding RNAs) and will be discussed further below. Previously labelled "junk DNA", the non-coding RNAs are increasingly recognized as key regulators in several aspects to health and disease, including cancer.

Each of the epigenetic levels can be influenced through inherited genetic information, through external exposures and ongoing biological processes, such as aging. Epigenetic changes may lead to cancer development. Notably, altered genetic information in the cancer itself may also promote epigenetic changes (Fig. 1**)** and further development of tumour heterogeneity. This may also be influenced further by the effects of chemotherapy [2], leading to epigenetic changes or selection of subclones that bears particular epigenetic marks. This adds complexity to the understanding and research into epigenetics in cancer yet provides opportunities for finding novel therapeutic targets.

Several classes of epigenetic regulators (Fig. 3) exist and they are broadly defined as 'writers', 'readers' and 'erasers' [3,19,20]. As epigenetic alterations are reversible, inhibitors targeting the epigenetic processes may be promising anticancer strategies. Briefly, regulators that write the marks are known as 'writers' and include DNA methyltransferases, histone methyltransferases, and histone acetyltransferases. 'Readers' are, as derived from the name, reading the marks and include regulators such as the bromodomain, chromodomain, and tudor proteins. Lastly, regulators that can erase marks are called 'erasers', and among these are examples such as histone deacetylases (HDAC) and histone demethylases

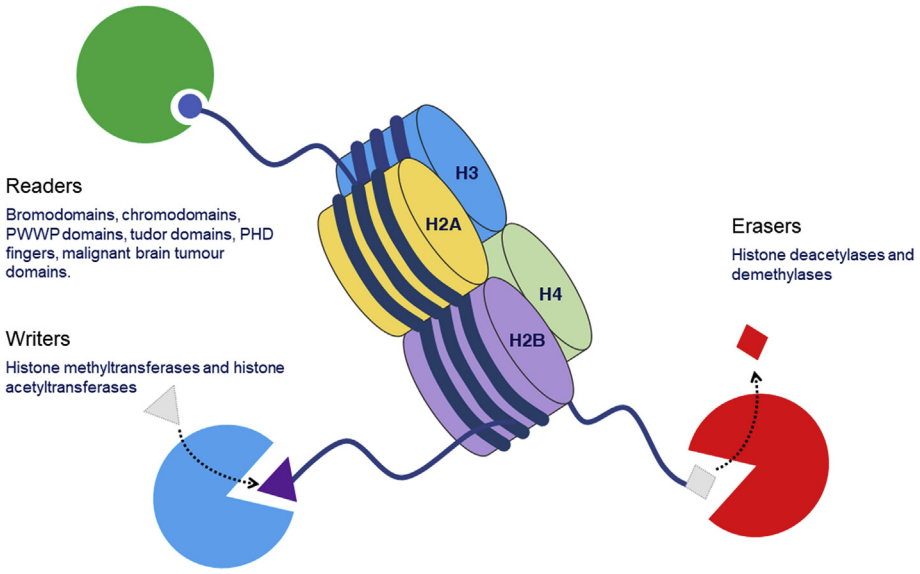

Fig. 3. Epigenetic regulation through readers, writers and erasers.
Regulators that write the marks are known as 'writers' and include DNA methyltransferases, histone methyltransferases, and histone acetyltransferases. 'Readers' are reading the marks and include regulators such as the bromodomain, chromodomain, and tudor proteins. Regulators that can erase marks are called 'erasers', and among these are examples such as histone deacetylases (HDAC) and histone demethylases (HDM); and remodellers of the chromatin, such as components of the SWI/SNF (SWItch/Sucrose Non-Fermentable) nucleosome remodelling complex. As epigenetic alterations are reversible, inhibitors targeting the epigenetic processes may be promising anticancer strategies.

(HDM); and remodellers of the chromatin, such as components of the SWI/SNF (SWItch/Sucrose Non-Fermentable) nucleosome remodelling complex [20,21]. Mutation of specific epigenetic modifiers occurs frequently in a variety of cancers demonstrates that altered epigenetic regulation may play an important role in cancer development (Fig. 1), yet may also be a bystander effect of carcinogenesis itself [22].

The best-known and most explored epigenetic alteration is DNA methylation [23,24] (Fig. 4). DNA methylation has critical roles in the control of gene activity and the architecture of the nucleus of the cell. Histones serve as molecular structures that participate in the regulation of gene expression. Consequently, chemical modification of histones may alter gene expression. Histones contribute to epigenetic post-translational modifications through lysine acetylation, arginine and lysine methylation, and serine phosphorylation - modifications that affect gene transcription and DNA repair [25]. In humans, DNA methylation occurs in CpGs (Fig. 4). Regions in the DNA that contain many adjacent cytosine and guanine nucleotides are called 'CpG-islands' (… CGCGCGCGCG ….). CpG islands are not randomly distributed in the genome but rather often found in the regulatory region of many genes (approximately 40% of the promoters of human genes). These islands are usually not methylated in normal cells. While CpG islands are usually unmethylated in normal cells, and the genes downstream of these unmethylated promoters are transcribed in the presence of transcriptional activators, genomic platforms have confirmed that almost 10% of normally unmethylated promoter CpG islands, many of them belonging to tumour suppressor genes, become abnormally methylated and thus silenced in cancer [26,27] promoting carcinogenesis. In colorectal cancer [15,28], a CpG-island methylator phenotype (CIMP) has been recognized as a separate molecular group (besides microsatellite instable and chromosomal instable tumours) that has distinct clinical and pathological characteristics [29]. For one, prognosis is demonstrated to be significantly worse for the CIMP positive group in both colorectal [30] and hepatocellular carcinoma [31].

Heterochromatin is a closed chromatin conformation that is often associated with DNA methylation and inactive gene transcription. In contrast, the euchromatin state is in an open conformation and associates with active gene transcription, presumably secondary to increased transcription factor binding. DNA methyltransferases and Methyl-CpG-Binding Domain proteins (MBDs) work with histone-modifying enzymes for regulating all DNA-templated processes including transcription, repair, replication, and recombination [23,26,32]. Histone N-terminal tails can undergo many chemical modifications, including acetylation, methylation, phosphorylation, and ubiquitination (Fig. 5).

Depending on the combination of modifications in a specific genomic region, chromatin remains more or less packed, blocking or permitting the nuclear processes. Importantly, this histone code is not static but rather is changing in a context-dependent manner. As such, it can both facilitate or repress gene transcription. Histone modifications are still being discovered and are found in novel combinations, highlighting the flux of knowledge in this field. The influence of these marks on other histone modifications is referred to as "histone crosstalk" and is of crucial importance for the transcriptional readout of a gene [21,26]. The various enzymes responsible for such modifications throughout histone tails include histone acetyltransferases (HATs), multiple classes of histone deacetylases (HDAC classes I, II, III and IV) and sirtuins (a HDAC class III, which are NAD + dependent), histone methyltransferases (HMTs), histone demethylases (HDMs), histone kinases and phosphatases, histone ubiquitin ligases, and deubiquitinases (as reviewed in detail in Ref. [26]). Consistently, with the fact that disruption of normal patterns of covalent histone modifications is a defined cancer hallmark many such enzymes that add or remove (writers and erasers) these chemical groups, and also those that recognize them (readers) are mutated or misregulated in cancer (see Fig. 3).

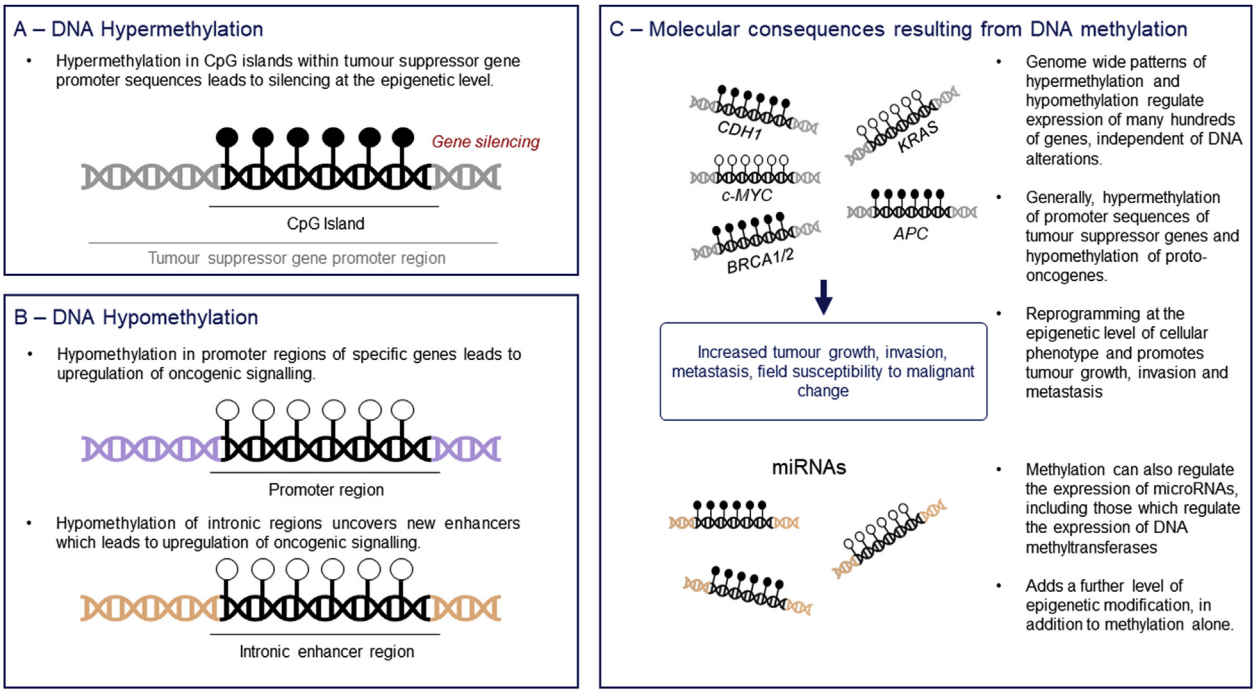

Fig. 4. Overview of DNA methylation and effects.
DNA methylation occurs in the context of chemical modifications of histone proteins by the addition of a methyl group to DNA at the 5-carbon of the cytosine pyrimidine ring that precedes a guanine; these are called dinucleotide CpGs. Regions in the DNA that contain many adjacent cytosine and guanine nucleotides are called 'CpG-islands'. The "p" in CpG refers to the phosphodiester bond between the cytosine and the guanine.

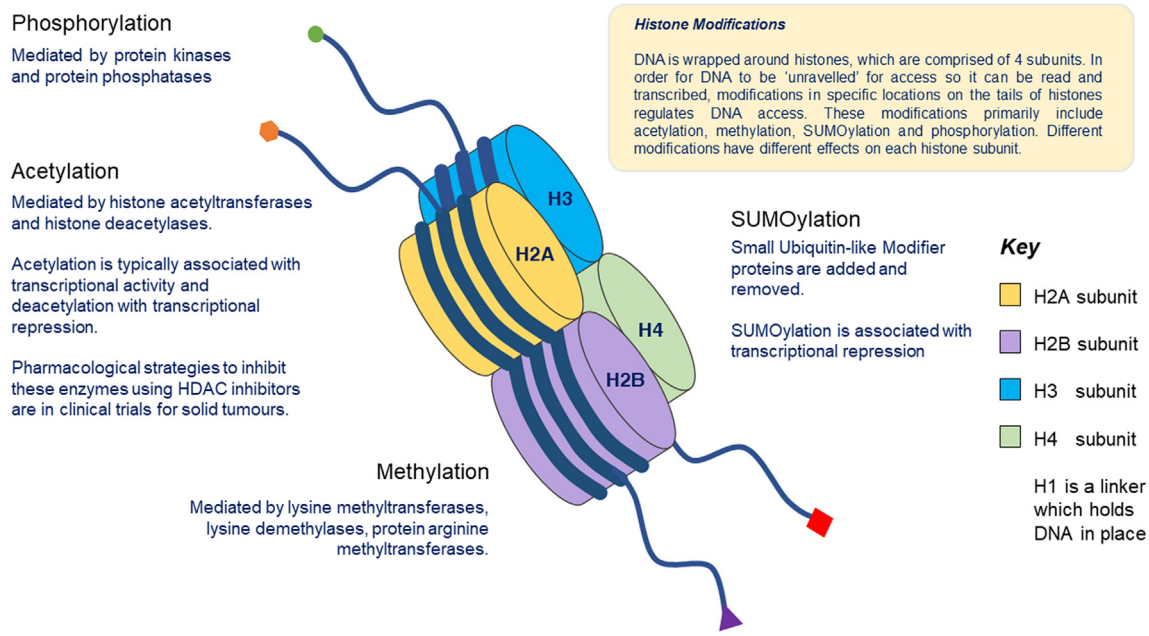

Fig. 5. Overview of histone modifications. Histone modifications regulate access to DNA and have a major role in the reading, transcription and alteration of DNA. Different tail modifications result in differential effects on the accessibility and stability of DNA packed around histones.

The sequences of the human genome that are protein-coding account for only 2% of the entire DNA, yet are the most thoroughly investigated parts of the genome. The remaining part of the genome (that is, the part not coding for proteins) is made up of noncoding RNAs (ncRNAs) [17,18]. Previously dubbed 'junk DNA' due to the believed lack of function, the non-coding part of DNA is now demonstrated to be involved in anything from embryogenesis to cancer development. As such, ncRNAs include microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), small interfering RNAs (siRNAs), and long noncoding RNAs (lncRNAs). While miRNAs are probably the most investigated and best understood of these in cancer [33,34], there is an increasing interest in understanding the other ncRNAs as therapeutic strategies may be developed to counteract these perturbations [17]. The ncRNAs are epigenetically regulated in carcinogenesis and metastases development and consequently sculpting the epigenetic profile of a cancer cell. Consequently, ncRNA are modulating the expression of other RNA molecules too [17,33] and by that not only affect the DNA methylation status of certain genomic loci but also interact with histone-modifying complexes, changing the structure of the chromatin itself [17].

Epigenetics alterations from cradle to grave

Epigenetic marks change during foetal development (thus, turning mechanisms on/off), through adult life (through various exposures), and occur throughout the aging process [4,35–38], Fig. 1. Epigenetics are influenced by a number of external and internal exposures, such as physical activity and nutrition [6,39,40]. Some changes play an important role in the establishment and regulation of gene programs, but others seem to occur without any apparent known physiological role. Nutritional patterns, physical activity and medications can affect the epigenome [35,41]. The potential use of natural epigenetic modifiers in the chemoprevention of cancer is increasingly explored and may provide opportunities for lifestyle intervention and for prevention of cancer [42].

The way in which energy is used in cells is determined under the influence of environmental factors such as nutritional availability [43]. Metabolic adaptation is mainly achieved through the modulation of gene expression [44,45], and may also involve epigenetic mechanisms that enable long-term regulation. Nutrients and their metabolites may influence on the epigenome through acting as substrates or as coenzymes for epigenetic-modifying enzymes, while other epigenetic regulators may influence metabolic genes in a way that lead to a shift in energy flow. These findings suggest the concept of metabolism-epigenome crosstalk that may contribute to the formation of a long-term metabolic phenotype [43]. Epigenetic regulation of metabolism [46] is relevant to understanding the development of obesity and pathogenesis of related metabolic disorders. Epigenetic alterations that occur through nutritional conditions and microbial exposures before or after birth may affect disease risk in adulthood [47] – decades after the metabolic events took place (Fig. 1).

Age-dependent loss of global methylation, together with hypermethylation of CpG islands associated with cancer-related genes, may be influenced by nutritional and metabolic factors [48]. Several compounds of nutrition, such as folates and vitamins, are essential for the maintenance of normal DNA methylation. Folate metabolism is known to modify epigenetic mechanisms under experimental conditions, and more recent findings has explored the important roles of vitamin C and D in maintenance of the epigenome [49,50]. Further, most cancer cells exploit metabolic pathways for their hyperproliferative activity [51], while metabolic reprogramming leads to aberrant epigenetic regulation in some cancers [52]. Thus, epigenetics influences human cells in a number of ways, from conception and in the prenatal phase, through birth and long into aging. From a public health perspective, a better understanding may open avenues into preventive strategies and early detection or risk through epigenetic mechanisms, markers and profiles.

Epigenetics in cancer evolution

Genome-wide sequencing has shown that all cancers have 1000s of somatic genetic and epigenetic alterations that are not present in the patient's germline genome. Notably, only a very

small number of these alterations are in 'driver genes' associated with a growth advantage of the cancer cell over other normal cells in the surroundings [13]. The remaining alterations are 'passengers' found in tumour cells only because they occurred coincidentally during the long march toward carcinogenesis. Of the more than 20,000 genes in the human genome, only some 200 have been shown to act as driver genes for common cancers [13,53]. What is more, these genes appear to function through a limited number of cancer pathways.

Notably, tumours evolve in three broad phases – the breakthrough, expansion and invasive phases [53] (Fig. 1). In the breakthrough phase, a cell acquires a driver-gene mutation and begins to proliferate abnormally [53]. Known cancer mutation rates suggest that these further mutations are unlikely to occur without a large increase in cell number during the breakthrough phase [13]. The mutation initiating the breakthrough phase is often very specific — a limited number of growth-regulating pathways seem able to initiate neoplasia in a given cell type [54]. As tumours progress, this specificity seems to be progressively lost, so a greater number of driver genes can transform a cell from the expansion phase to the invasive phase. Knowing that so few genetic mutations are required in neoplastic transformation could possibly be explained by the added influence of epigenetic alterations [13].

The interaction between the genome and epigenome throughout the process of carcinogenesis is not fully understood [9]. Epigenetic reprograming of neoplastic cells have been proposed [55,56], with the idea that the epigenome and genome interact synergistically to evolve to stressors to ensure survival (Fig. 1). Epigenetic regulation provides a degree of plasticity, thus granting cancer cells the ability to repress the expression of specific genes in response to stresses and stimuli. An example of this is in cancer metabolism, where dynamic changes in oxygen tension or nutrients leads to epigenetic reprogramming, thus enabling cell survival and evolution. Furthermore, cancer cells may acquire mutations in genes coding specific transcription factor drivers which modulate collaborating chromatin regulators, thus dynamically regulate their epigenetic circuits to rewire differentiated cancer cells into stem-like cells, leading to upregulation of cancer growth [55]. This acquisition of stem-like characteristics is linked with the development of metastasis and spread of disease, as cells have greater plasticity to move, migrate and survive. Indeed, the metastatic process is a complex and dynamic process consisting of numerous steps and interactions [56–59] – even among the cancers cells that reach the circulation it is estimated that less than 1 in 10000 may have a metastatic potential. Thus, genetic mutations may not be a causal factor for the transition from primary tumour to metastatic lesion. Rather, as epigenetic changes are dynamic they may play an important role in determining metastatic phenotypes [56,57]. In an analogy to the driver and passenger mutations mentioned above, concepts have now emerged for "driver epigenetic" events that may influence the metastatic potential. Such 'epi-driver' and 'epi-passenger' events in metastasis may provide for better understanding of the biological processes and also provide for new areas for therapeutic targets [56,57,60]. Taken together, the epigenome cooperates with the genome as cancers develop and eventually metastasize [56,57] with the potential for exploring novel biomarkers of risk or monitoring of disease and, drugable targets for therapy.

Potential clinical implications of epigenetics

Several classes of epigenetic regulators of writers, readers, and erasers have been implicated in mechanisms leading to intra-tumoural heterogeneity and chemotherapy resistance [61–63]. The clinical implications are multifold and is likely to affect how we approach cancer therapies in the future. For example, the ability to biopsy tumour changes in subclones at time of diagnosis and during follow up may help tailor therapy to the epigenetic and genetic makeup of the tumour [54]. Further, epigenetic marks may be used as predictive or prognostic biomarker of treatment success, e.g. for measure of response or as indicator of recurrence. Finally, specific epigenetic drugs may be used together with conventional drugs to achieve a more tailored and specific response to the specific cancer under treatment [20,57,60,64,65].

Epigenetic therapies, in which the goal is to reverse these changes, are now in routine clinical use for haematological malignancies [12,66]. The application of epigenetic therapies in the treatment of solid tumours is also emerging as a potential therapeutic option with multiple phase I and II studies underway (Table 1) [20,67]. The limitations posed by cancer treatments involve (among others) the unintended epigenetic modifications that may result in exacerbation of tumour progression – a side effect that clearly would contradict the use of epigenetic therapy for curative purposes. The specificity restrictions (i.e. tumour specific effect not involving the normal epigenome) posed by epigenetic therapies and ways to address such limitations is presented in detail elsewhere [68]. Further, with the next generation of targets and drugs, there is hope that novel epigenetic therapies may improve drug targeting and drug delivery, optimize dosing schedules, and improve the efficacy of pre-existing treatment modalities, such as chemotherapy, radiation, and immunotherapy [69].

Classification and use of epigenetic biomarkers

Tumours have in the past been classified based on their (likely) tissue of origin and differentiation and grade. This still represents the mainstay for diagnosis and prognosis, but increasingly epigenetic features help classify tumours into distinct therapeutic and prognostic classes.

The ability to identify high- and low-risk patients based on circulating or tissue biomarkers in cancer is still poor. Molecular biology has, over the years, given insight into basic principles of cancer initiation and development and increasingly these are being exploited as biomarkers, and several epigenetic markers or tools have been proposed in various tumours. This include aberrations increasing risk of tumour development, (epi-) genetic changes associated with the stepwise progression of the disease, and errors predicting response to a specific treatment. Because several epigenetic changes occur before histopathological changes are present, they can serve as biomarkers for cancer diagnosis and risk assessment [70]. Many cancers may remain asymptomatic until relatively late stages; in managing the disease, efforts should be focused on early detection, accurate prediction of disease progression, and frequent monitoring [25]. Based on epigenomic information, biomarkers have been identified that may serve as diagnostic tools; some such biomarkers may also be useful in identifying individuals who will respond to therapy and, potentially, live longer [15,25,27,59,70–72]. Recently, in a multicentre study the investigators were able to predict a primary cancer of origin in 87% of 216 patients who had a cancer with unknown primary by using DNA methylation profiling [73]. Determining the epigenetic landscape of tumours is key to the success of this and has been performed in several tumour types, some of which are presented as examples in paragraphs below.

One area in which epigenetics has demonstrated considerable promise is in the development of liquid cancer biopsies [74]. Examination of methylation patterns on cell-free DNA or circulating tumour DNA has been proposed in multiple malignancies as minimally invasive diagnostic cancer test. These tests work in a variety of ways and are typically based on PCR amplification assays,

Table 1
Summary of ongoing trials of epigenetic therapies.

Authors	Year	Compound	Phase	Status	Target	Disease	Recruiting?
Azad et al.	2013	Azacitidine with nab-Paclitaxel and Gemcitabine	II	Recruiting to March 2018 (last update)	Azacitidine- Inhibition of DNA methyltransferase	Adjuvant therapy for resected pancreatic cancer	Active recruitment ongoing
Hellmann et al.	2017	Guadecitabine and Mocetinostat with Pembrolizumab	I	Recruiting to June 2018 (last update)	Guadecitabine - Inhibition of DNA methyltransferase Mocetinostat - Histone deacetylase inhibitor	Stage IIIb or IV non-small cell lung cancer	Active recruitment ongoing
Azad et al.	2015	Romidepsin, Azacitidine (CC-486) and Pembrolizumab (MK-3475)	I	Recruiting to March 2018 (last update)	Romidepsin – Histone deacetylase inhibitor Azacitidine- Inhibition of DNA methyltransferase	Microsatellite stable metastatic colorectal cancer having had first line treatment	Active recruitment ongoing
Velcheti et al.	2016	Decitabine, Tetrahydrouridine, Nivolumab	II	Recruiting to December 2017 (last update)	Decitabine - hibition of DNA methyltransferase Tetrahydrouridine - Inhibitor of cytidine deaminase	Non-small cell lung cancer having had first line treatment	Active recruitment ongoing
Munster et al.	2015	Vorinostat, Pembrolizumab, Tamoxifen	II	Recruiting to March 2018 (last update)	Vorinostat - Histone deacetylase inhibitor	Pre and postmenopausal women or men with stage IV breast cancer	Active recruitment ongoing
Shah et al.	2011	Azacitidine, Oxaliplatin, Epirubicin, Capecitabine	I	Recruited and results expected November 2018	Azacitidine- Inhibition of DNA methyltransferase	Resectable junctional gastroesophageal adenocarcinoma or poorly differentiated carcinoma	Active, not recruiting
Brahmer et al.	2013	Azacitidine, Entinostat, Nivolumab	II	Recruiting to October 2017 (last update)	Azacitidine- Inhibition of DNA methyltransferase Entinostat - Histone deacetylase inhibitor	Stage IIIB, IV or recurrent non-small cell lung cancer	Active recruitment ongoing
Fandi et al.	2015	Azacitidine or placebo, Pembrolizumab	II	Recruited and first results posted May 2018	Azacitidine- Inhibition of DNA methyltransferase	Stage IIIB, IV or recurrent non-small cell lung cancer	First results posted
Yip et al.	2018	Phenelzine Sulfate and Nanoparticle albumin-bound Paclitaxel	I	Recruiting to April 2018 (last update)	Phenelzine Sulfate - Inhibitor of Lysine-specific histone demethylase 1	Metastatic or locally advanced, inoperable breast cancer	Active recruitment ongoing
Preskitt et al.	2016	Curcumin and 5-Fluorouracil	I	Unknown	Curcumin - Histone deacetylase inhibitor	Metastatic colon cancer	Unknown
Plimack et al.	2017	Guadecitabine and Atezolizumab	II	Recruiting to June 2018 (last update)	Guadecitabine - Inhibition of DNA methyltransferase	Checkpoint inhibitor refractory or resistant urothelial carcinoma	Active recruitment ongoing
Shu et al.	2017	Guadecitabine (SGI-110) and Durvalumab or Tremelimumab	I	Recruiting to June 2018 (last update)	Guadecitabine - Inhibition of DNA methyltransferase	Small cell lung cancer	Active recruitment ongoing
Doroshow et al.	2015	Deoxycytidine (TyCyd)	I	Recruiting to July 2018 (last update)	Deoxycytidine - Inhibition of DNA methyltransferase	Patients with treatment refractory advanced solid tumours	Active recruitment ongoing
O'Hayer	2016	Azacitidine or INCB057643 or INCB059872 and Epacadostat with Pembrolizumab	I/II	Not recruiting (last update June 2018)	Azacitidine- Inhibition of DNA methyltransferase Epacadostat - Histone deacetylase inhibitor INCB057643- Bromodomain and extra-terminal (BET) inhibitor INCB059872- Inhibitor of Lysine-specific histone demethylase 1	Advanced solid tumours	Active, not recruiting

Examples of active trials updated in past 4 years (until December 2018) on ClinicalTrials.gov testing epigenetic therapies in solid tumours.

next-generation sequencing or on the physical properties of DNA itself. Simply, they work to identify cancer-specific methylation patterns at specific loci. A range of bodily fluids may be used in this way, with blood and urine liquid biopsy tests currently in advanced development [72]. The performance of these methylation-based assays has a significant advantage over genomic cell free DNA testing. In early stage or low-grade disease, methylation patterns are more prominent than the low number of genomic mutations that occur early in tumour development [73]. So far, methylation based liquid biopsy has been investigated in multiple malignancies, including breast, colorectal, pancreas and lung [74–76]. Results and quality of these studies have been variable, but the specificities and sensitivities are promising in the populations they were tested in – typically around 90%. One of the more challenging questions for liquid biopsies is where these will be of most use clinically, but there are several useful roles to both diagnostic, prognostic and predictive biomarker use [74,75]. There are likely to be roles for epigenetic biomarkers in early detection and follow-up, however, a universal test for all cancers which would have the most utility in a public health setting remains someway off. However, a recent multipanel test in >1000 patients showed considerable potential for early diagnosis and detection of cancer at a time when the disease would be potentially curable (by surgery) as tested across a spectrum of cancer types [76]. Furthermore, liquid biopsy using methylation assays provides limited information about tumour biology and whether these tests could enable precision medicine in

a neo-adjuvant setting without formal biopsy is unknown.

Colorectal cancer

For the assessment of primary colorectal tumours there are at least 3 suggested classifications that comes into consideration [15]. One is a hyper mutated group that includes defective DNA mismatch repair with microsatellite instability and POLE (DNA polymerase epsilon) mutations (about ~15% of CRC patients), containing multiple frameshifted genes and $BRAF^{V600E}$. The second is a non-hyper mutated group with multiple somatic copy number alterations and aneuploidy, previously known as chromosomal instability (CIN) type of tumours (in ~85%), containing oncogenic activation of KRAS and PIK3CA and mutation and loss of heterozygosity of tumour suppressor genes, such as APC and TP53. A third group is named CpG island methylator phenotype (CIMP) type CRCs (in ~20%) that overlap greatly with microsatellite instability CRCs and some non-hyper mutated CRCs [77]. CIMP tumours have methylated CpG islands and epigenetic alterations are essential in these cancers. Lastly a fourth group (or, a modifier group) is named after Elevated Microsatellite Alterations at Selected tetranucleotide (EMAST) repeats (found in up to ~60% of CRC, but also in a range of other cancer types [14]) that associates with metastatic behaviour in both hyper mutated and non-hyper mutated groups.

Components from these classifications are now used as diagnostic, prognostic, and treatment biomarkers [15,78], yet universal agreement on such a new classification has not been reached with alternative proposals published [79,80] and several negative studies exist [81]. However, several studies have also reported lack of a clinical role of epigenetic markers so further work needs to be done to refine the role of epigenetics in the clinical management of CRC.

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) has an extremely poor prognosis, with limited effective treatments. Alterations in epigenetic regulation are frequently found in PDAC [82], particularly in the regulation of genes involved with oncogenic signalling, with metabolic alterations and, in the metastatic process [45,83–85]. The epigenetic landscape of PDAC may be broadly classified into 3 groups; basal-like tumours with enhancers across several important oncogenic signalling pathways, 'classical' tumours with enhancers (pancreatic development genes) and 'classical' tumours with active promoters across similar regions as the second group. The activity of epigenetic regulators in pancreatic cancer, particularly the first subgroup, is associated with upregulation of genes responsible for aggressive tumour biology (EGFR, ErbB), deregulation of cell differentiation (YAP1, MYC, E2F7, HEY1) and promotion of metastasis through epithelial mesenchymal transition (HIPPO, WNT family, TGFβ). Epigenetic regulation across all these groups was found to silence tumour suppressor genes. Several compounds have been trialled, targeted to epigenetic alterations in tumours, including trials of curcumin (a p300 histone acetyltransferase inhibitor) and Histone deacetylase inhibitors (HDACi) such as Vorinostat [86]. Some patients have demonstrated response to these therapies but much more research is required to draw meaningful conclusions. It is currently hoped that immunotherapy and epigenetic targets, with or without conventional chemotherapy, may enhance response rates and effect in patients with PDAC. A detailed overview of ongoing research, trial sand mechanisms is provided elsewhere [86].

Epigenetic alterations in pancreatic cancer, in particular cell-free DNA and measurement of DNA methylation in pancreatic juice, offers a minimally invasive approach to diagnostics and prognostication. Panels of epigenetic biomarkers have been demonstrated to achieve sensitivities and specificities of 80 to upwards of 90%, however, these studies lack meaningful validation thus precluding their use in routine practice [87,88]. However, recent panels have showed promise for liquid biopsy technology as a pre-diagnostic screening tool for patient with PDAC.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) has a complex aetiology, typically evolving off the background of inflammatory liver diseases. Multiple integrative analyses of HCC have uncovered a complex landscape of three, distinct molecular subtypes of HCC. The first appears to be associated with normal body weight, Asian ethnicity and hepatitis B virus infection, whereas the second and third subtypes harbour mutations across CTNNB1, TERT promoters and, for the third subtype, in the TP53 gene. In HCC, there are several levels of epigenetic dysregulation involved in carcinogenesis and the stepwise progression towards metastatic or incurable disease [31,58,89,90]. For one, the CDKN2A tumour suppressor gene is found to be frequently silenced by DNA hypermethylation [91]. Further epigenetic alterations are found in the third subtype of HCC which lead to chromosomal and genome instability, in particular, the hypomethylation of multiple CpG sites [89]. Micro-RNA-122 (miR-122) expression is found to be aberrant in IDH1/2 mutant HCC, with reduced expression throughout tumour tissues which is associated with poor survival. It is thought miR-122 has tumour suppressor functions by modulating the effects of TP53 through Mdm2 [92,93]. Study of the methylation status and copy number variation found in HCC identified aberrant DNA methylation and copy number alteration was significantly associated with poorer survival, although this observation was not further developed as a tool for prognostication. Circulating tumour DNA methylation has found to be an accurate test, with very high sensitivity and specificity for detecting HCC and aiding in treatment stratification [94]. Little in the way of clinical trials of agents targeting epigenetic alterations have been performed for HCC, however, the concomitant use of HDAC inhibitors alongside Sorafenib has been explored with some promising results in small trials [95,96].

Breast cancer

Compared to other tumour types, the epigenetics of breast cancer are relatively well characterized and are known to play an important role in disease behaviour. Epigenetic drivers are found early in breast cancer carcinogenesis, including early DNA methylation and alteration in chromatin states [16]. Furthermore, lncRNAs and miRNAs have been found to affect wide numbers of genes, regulating their function and driving carcinogenesis and the development of intratumoural heterogeneity. Throughout the evolution of breast cancer, epigenetic reprogramming of these tumours occurs and have been found on the APC, CDH1 and CTNNB1 genes. Distinct patterns of reprogramming have been found through from ductal hyperplasia, ductal carcinoma in-situ to invasive carcinoma. Interestingly, the finding of distinct methylation patterns is not just confined to malignant breast cancer cells [97]. Adjacent, histopathologically normal, tissue has been found to demonstrate similar DNA methylation profiles to cancerous cells revealing a field effect. Epigenetic profiling of tumours and circulating DNA has raised the possibility that measurement of DNA methylation may be used to predict survival clinically. So far, however, few of these have made it to clinical practice and or trials of epigenetic alterations to guide cancer therapy [98,99]. As with other tumour types, HDAC inhibitors have been trialled in breast cancer, with varying degrees of success and at present there is a lack of convincing placebo-controlled data from a large trial to support routine clinical use [100,101].

Therapies targeting DNA methylation have also been trialled in breast cancer, DNA methyltransferases inhibitors (known as

DNMTs) remove methyl groups from DNA [102,103]. They are used in haematological malignancies, however, their use in solid tumours has not progressed past clinical trials due to their poor side-effect profiles [104]. The compounds, Azacitidine, Decitabine and Zebularine typically act by forming covalent bonds with DNA methyltransferases and thus cause them to become 'trapped' and unable to methylate DNA further. To date, trials have been disappointing, with very limited clinical effects demonstrated from these therapies.

Potential research caveats in epigenetics

Generalisability and reproducibility in using epigenetic markers for classification and diagnosis has been hampered by the lack of standardized and unified protocols and analytical designs. For example, we found in a previous study that the call of CIMP classification would deviate substantially between cases depending on what definitions, genes and panels where used for defining CIMP status [105]. A total of 16 different definitions of CIMP were identified in a systematic review [106]. In that paper, all studies on CRC prognosis according to the various definitions of CIMP were searched for and some 36 studies were identified [106]. Of the 36 studies, 30 (83%) reported the association of CIMP and CRC prognosis and 11 (31%) reported the association of CIMP with survival after chemotherapy. Most studies reported a poorer prognosis for patients with CIMP-positive CRC than with CIMP-negative CRC. Inconsistent results or varying effect strengths could not be explained by different CIMP definitions used. Response to specific therapies according to CIMP status was inconsistent across studies. As the authors conclude, comparative analyses of different CIMP panels in the same large study populations are needed to further clarify the role of CIMP definitions and to find out how methylation information can best be used to predict CRC prognosis and response to specific CRC therapies. From both studies [105,106] goes the notion that better standardization and agreement between studies are needed.

Future research

Although the human genome has been well characterized, along with somatic mutational drivers of cancer, relatively little is known about how various epigenetic alterations interact with one another and the genome. The landscape of DNA methylation, micro-RNAs and to some extent long non-coding RNAs have been studied with next-generation approaches in initiatives such as The Cancer Genome Atlas, however other epigenetic elements remain poorly characterized.

Modelling how epigenetics interacts with the cancer genome and subsequently the transcriptome will be key to effective treatment stratification and identifying how epigenetic alterations can be therapeutically targeted. This may be achieved in multiple ways, however, use of computational *in-silico* methods, including machine learning, may lend themselves well to identifying potential interactions which may then be explored in high-throughput *in-vitro* and *in-vivo* studies. These computational approaches are highly complex as they are required not only to analyse genome wide data, but also to model the complex interactions across pathways and at multiple levels of the epigenome. Technologies such as CRISPR-Cas9 and Cre-lox recombination could be used to accomplish this. These technologies permit efficient editing of some nucleic acid components of the epigenome and may be turned on or off using elements dependent on external stimuli provided by researchers.

Detection of aberrant DNA methylation is being developed as biomarkers for prognostic and diagnostic purposes in gastrointestinal cancers [107]. Novel ways of obtaining samples, such as liquid biopsies, may lead to less invasive and more clinical useful tests in the future [74]. Prognostication studies based on epigenetic elements are published [81], but few have made it into routine clinical practice. An example of a commonly used epigenetic test is measurement of the methylation status of the MGMT (O-6-methylguanine-DNA methyltransferase) gene in glioblastoma. Methylation of this gene, a key protein responsible for maintaining genome stability through mismatch repair, guides use of Temozolomide therapy, with patients having methylation of the MGMT promoter having better responses to therapy. The careful validation and study of this through molecular studies and into randomized control trials has enabled this epigenetic marker to reach the clinic and improve cancer outcomes for patients. However, many epigenetic prognostication studies lack this approach, commonly have low sensitivity and specificity for isolated markers, and do not consider a 'multi-omic' approach. To have clinical utility, researchers must be careful not to consider the epigenome in isolation, as its primary role appears to be regulation of the genome and transcriptome. Recent developments in technology, such as single-cell omics technology [108] tries to overcome these hurdles and may have a greater clinical impact in the near future. Lastly, recognition of variation in exposure and how epigenetic regulation across global populations may drive risk, influence cancer phenotype and potentially present with different outcomes and responses to therapies is necessary [109]. This requires investigation across diverse populations and from various regions of the world for valid and robust results.

Conclusion

The epigenetic regulation of cancer is complex and not yet fully understood. Several mechanisms of epigenetic regulation exist and already are known to have implications for cancer therapies and disease prognostication. Surgical oncologists must be aware of recent developments in the area to understand the application of epigenetics to clinical practice and to improve research to benefit patients.

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