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Epigenomic technologies for precision oncology

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ABSTRACT

Epigenetic patterns in a cell control the expression of genes and consequently determine the phenotype of a cell. Cancer cells possess altered epigenomes which include aberrant patterns of DNA methylation, histone tail modifications, nucleosome positioning and of the three-dimensional chromatin organization within a nucleus. These altered epigenetic patterns are potential useful biomarkers to detect cancer cells and to classify tumor types. In addition, the cancer epigenome dictates the response of a cancer cell to therapeutic intervention and, therefore its knowledge, will allow to predict response to different therapeutic approaches. Here we review the current state-of-the-art technologies that have been developed to decipher epigenetic patterns on the genomic level and discuss how these methods are potentially useful for precision oncology.

1. Introduction

Every cell in the human body has the same genome, but different cell types possess distinct phenotypes driven by distinct gene expression patterns. These expression patterns are mediated by the activation of cell type-specific transcription factors (TFs) but also, in large parts, by a specific configuration of the epigenome: the interplay of DNA methylation, histone tail modifications, nucleosome positioning along the chromosomes and the 3D structure of the chromatin within the nucleus. Applying the latest next generation sequencing (NGS)-based technologies, it is now possible to map these epigenetic patterns within specific cell types and to track epigenetic programming in the context of cellular differentiation processes, or across various age groups. Unique and distinct epigenetic patterns of the chromatin drive cell type-specific expression programs. In neoplastic cells, these fine-tuned patterns are disturbed, resulting in dysregulated gene expression, including the silencing of tumor suppressor genes and the activation of cancer-related genes. Cancer-specific epigenetic patterns might also affect response to therapeutic interventions. Cancers originate from distinct stages of development or differentiation. These processes are associated with massive epigenetic programming and include stages at which cells might be 'epigenetically susceptible' to malignant transformation. Consequently, the epigenomes of cancer cells carry, in addition to cancer-specific alterations, marks that were preexisting in the cell-of-origin (Fig. 1). As a result, the identification of cancer cell-specific epigenome patterns requires sophisticated approaches that are based on global epigenetic profiling in small cell populations or even single cells and on sensitive *in silico* deconvolution techniques. Ultimately, the definition of cancer-specific epigenetic patterns will guide the identification of novel therapeutic targets and of novel diagnostic or predictive biomarkers. In this review we will focus on recent developments in the field of epigenomic profiling and their potential application to precision oncology.

1.1. Deciphering DNA modifications in cancer

Methylation of cytosine (mC) in the context of CG dinucleotides (CpG) is the most common DNA modification in mammals including humans. Most of the about 28 million CpGs in the human genome are methylated, while those located in the usually CpG-rich gene promoters are largely unmethylated (Fig. 2). Aberrant DNA methylation in tumor cells was an early observed epigenetic characteristic of cancers [1–3]. The prognostic relevance of tumor-specific DNA methylation patterns has been shown, for example, in chronic lymphocytic leukemia [4] and in juvenile myelomonocytic leukemia [5–7]. Moreover, smoking-associated DNA methylation patterns present in blood cells

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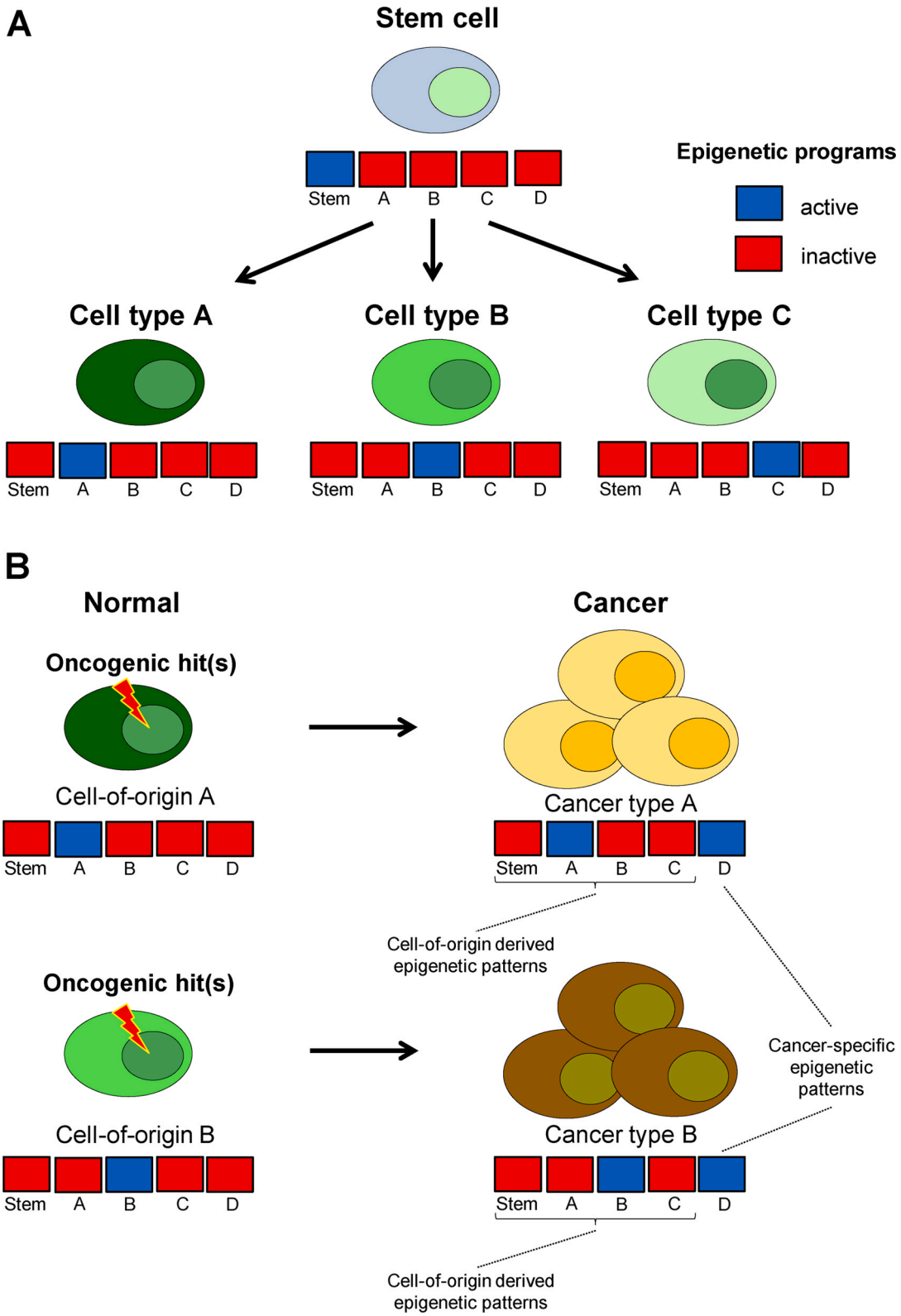

Fig. 1. The epigenomes of cancer cells. A) During differentiation, the epigenome is programmed in a cell type-specific manner. As a consequence, each cell has a cell type- and a differentiation stage-specific epigenetic landscape of active and inactive epigenetic programs. In addition, epigenetic patterns of normal cells are affected by their age and by (micro-)environmental conditions. The epigenome of a cell determines its phenotype by integrating these parameters, and, as a consequence, the cell's phenotype will slightly change over time. B) The effects mediated by an oncogenic hit will depend on the cell's phenotype and, hence, on its epigenome, resulting in different (sub-)types of cancer. The cancer cell-of-origin provides the epigenetic backbone of the cancer cell's epigenome, drives tumor biology, relates to prognosis and determines, how the disease responds to the cancer treatment applied. The cancer cell's epigenome can be decomposed into two main components: 1) the epigenome of the cell-of-origin, and 2) the cancer-specific epigenetic changes.

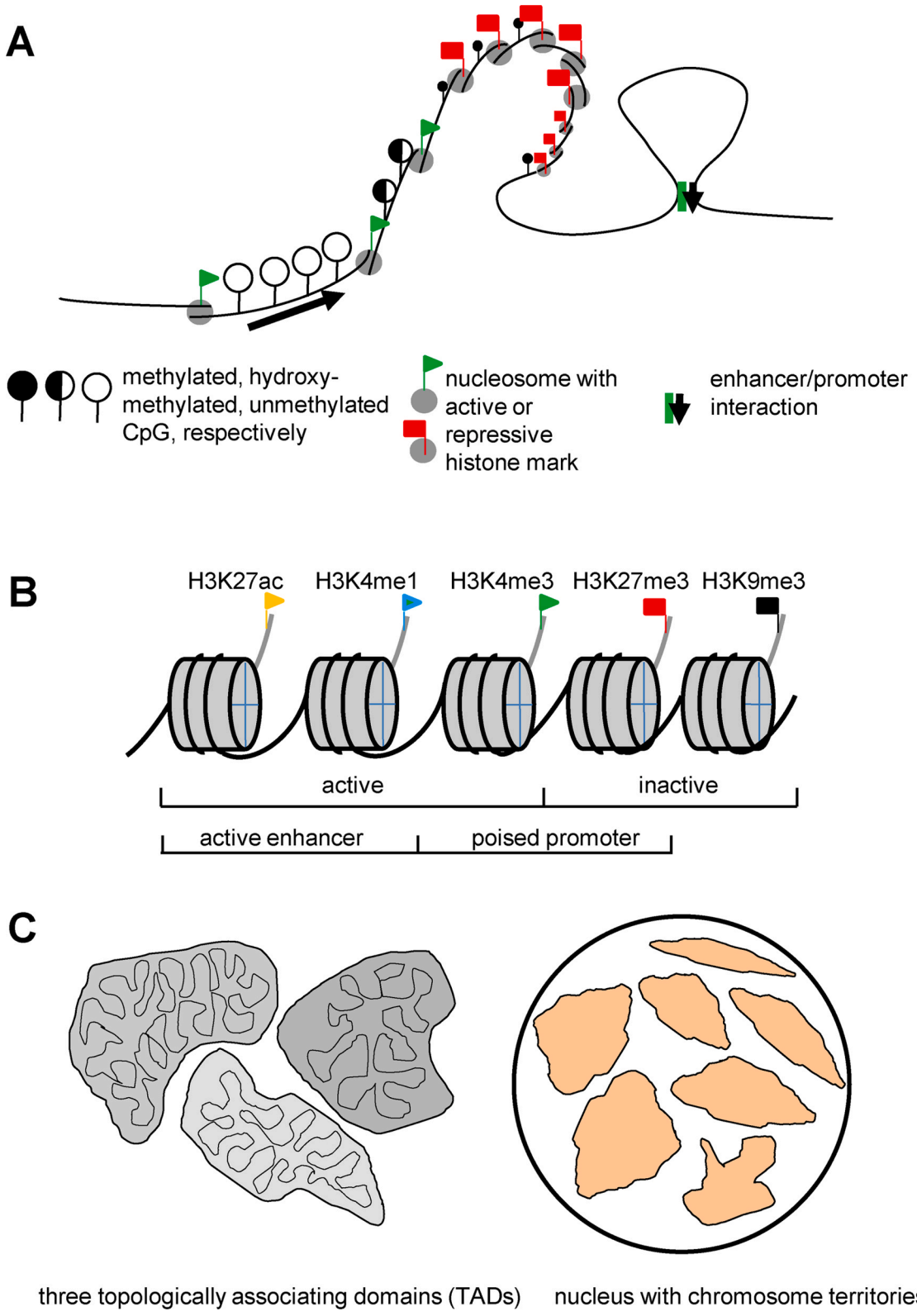

Fig. 2. Epigenomic features of the chromatin. A) The DNA methylome consists of methylated, hydroxymethylated and unmethylated CGs (black, black and white, white lollipops, respectively), the latter usually found in CpG-rich, active promoters (large arrow). The DNA is wrapped around nucleosomes (grey circles) which carry repressive (red flags) or activating (green flags) histone marks. Higher order looped structures juxtapose distant enhancers (green bar) with target promoters (short arrow) enabling assembly of the transcriptional machinery and initiation of transcription. B) Nucleosomes in open, active chromatin are less compacted and marked with H3K4ac, H3K4me1 and H3K4me3, while those in inactive chromatin are more densely packed and marked with H3K27me3 and H3K9me3. Active enhancers are enriched for H3K27ac and H3K4me1, and active promoters for H3K4me3. Bivalent promoters with both H3K4me3 and H3K27me3 marks are poised for transcription of developmental genes. C) The current model of the higher order chromatin structure and nucleus organization describes the genome as folded into different topologically associating domains (TADs) which are enriched for specific activating or inactivating chromatin marks. The nucleus is organized into chromosome territories, specific nuclear areas captured by individual chromosomes.

enabled to predict the incidence of lung cancer [8].

Current standard methods to analyze the DNA methylomes of cancer patients are whole genome bisulfite sequencing (WGBS) [9] which allows the interrogation of all CpG sites present in the genome and 850k EPIC methylation arrays (Illumina) which cover the DNA methylation levels of 850 000 preselected CpG dinucleotides in representative genomic areas [10] and can be applied even to DNA from formalin-fixed samples (Table 1). In contrast to WGBS, DNA methylation arrays are a simple, relatively low-cost technology that is suitable for large patient numbers. This technology was widely used in The Cancer Genome Atlas (TCGA) or in the International Cancer Genome Consortium (ICGC). Its applicability in routine diagnostics was recently demonstrated in a multicenter study which highlighted the classification of about 100 distinct tumor types affecting the central nervous system using a machine learning approach [11]. The study demonstrated high diagnostic precision of the technology and, furthermore, its diagnostic superiority in comparison to standard pathological diagnostics. A drawback is, however, that only a minority of all CpGs is covered by this array, resulting in a situation where DNA methylation levels of individual CpGs may undergo massive changes during normal cell differentiation or during tumorigenesis but cannot be detected since these CpGs are spared on the array. In contrast, the genome wide measurement of DNA methylation levels by WGBS allows a fine-grained discrimination between the intricate DNA methylation changes occurring during normal differentiation and cancer development [12] and, thus, may improve individual disease-staging and therapy. In general, WGBS proves advantageous in the identification of cancer-related methylation changes in enhancers which are highly cell type- and developmental stage-specific and, in contrast to promoters, CpG-poor [13,14]. However, WGBS comes with relatively high costs, around 1 000 Euro per sample, and the computational requirements regarding data storage, memory and bioinformatics tools and skills are demanding, making WGBS still quite a challenge in routine clinical diagnostics.

Gene regulatory elements are usually lowly methylated, and the levels of promoter methylation and transcription are frequently negatively correlated, while gene body methylation and transcription show a positive correlation [15,16]. Tumor-specific increases of methylation in promoters of tumor suppressor genes are used as biomarkers in precision oncology and analyzed in diverse malignancies to monitor onset, progression, recurrence and metastasis [17]. Local methylation changes can be identified routinely in a clinical setting by a variety of methods like pyrosequencing or matrix-assisted laser desorption ionization time of flight mass spectrometry [18].

Hydroxymethyl-cytosine (hmC) and the more oxidized, short-lived formyl- (fC) and carboxyl-cytosine (caC) are intermediates of active CpG demethylation (see Fig. 2) in the differentiation of normal cells but may also result in the epigenetic activation of oncogenes in cancer development. In glioblastoma, hmC is associated with cancer-related processes like stemness and proliferation, and low hmC levels correlate with poor prognosis [19]. hmC can be quantitated by methods like combined whole-genome bisulphite/oxidative bisulphite sequencing, EPIC arrays coupled with oxidative bisulphite treatment and antibody-based enrichment followed by sequencing. Each of these methods has its trade-offs with respect to detection limits, genomic coverage and costs [20]. Recent approaches enabling long-range genomic analysis like nanopore sequencing (see Table 1) now appear on the horizon to map DNA modifications based on the different DNA base chemistry [21]. However, nanopore sequencing is currently still too error prone to be used in routine cancer diagnostics.

1.2. Profiling chromatin accessibility and genomic targets of modified histones in cancer

The regional accessibility of the DNA for the transcriptional machinery is largely dictated by the positioning of nucleosomes in dependence of histone modifications (see Fig. 2) [22]. Integrated with

Table 1
Epigenetic methods in cancer diagnostics.

Epigenetic modification/target	Assay	Strength	Weakness	Ref.
Methyl (Hydroxymethyl)-cytosine	EPIC methylation array	Automatable, FFPE-DNA[a] compatible, cheap, simple evaluation	Low coverage, preselected sites	[10]
	WGBS[b]	Single CpG resolution	Computationally demanding, relatively expensive	[9]
	Single cell WGBS	Discriminates between individual cells	Low genomic coverage per cell	[56,57,58]
All cytosine modifications	Nanopore sequencing	Long reads, allelic resolution	Error prone, expensive	[21]
Chromatin accessibility	ATAC-seq[c]	Requires only low cell number, simple	May have sequence bias	[24,25]
	Single cell ATAC-seq	Discriminates between individual cells	Low genomic coverage per cell	[59,60]
Histone modifications, transcription factors, chromatin shaping proteins	Conventional ChIP-seq	Widely established protocol	Requires high cell numbers, low signal-to-background ratio, uncertain reproducibility	[31]
	ChIPmentation	Requires only low cell number, no library preparation, high-throughput compatible	Uncertain reproducibility	[35,36]
	CUT&RUN[d]	Requires only low cell number, high signal-to-background ratio	Requires library preparation	[37,38]
	CUT&Tag[e]/ACT-seq[f]	Requires only low cell number, high signal-to-background ratio, no library preparation, single cell compatible	Requires pA-Tn5ase protein not commercially available yet, may have sequence bias	[39,40]
Three dimensional chromatin structure/chromatin interaction	Hi-C	Genome wide coverage	Low resolution, computationally demanding	[46,47]
	Single cell Hi-C	Discriminates between individual cells	Low genomic coverage per cell	[61,62]
	Circular chromosome conformation assay (4C)	High resolution	May miss interactions, limited to viewpoint interactions	[55]

[a] Formalin-fixed paraffin embedded.
[b] Whole genome bisulfite sequencing.
[c] Assay for transposase-accessible chromatin using sequencing.
[d] Cleavage under targets and release using nuclease.
[e] Cleavage Under Targets and Tagmentation.
[f] Antibody-guided chromatin tagmentation.

additional genomic, epigenomic and transcriptomic features, profiles of the accessible chromatin provide essential insights into the mechanisms underlying the deregulation of the cancer genome [23]. Chromatin accessibility can be profiled by diverse approaches of which the assay for transposase-accessible chromatin using sequencing (ATAC-seq) [24,25] (see Table 1) is most favored because of its low input requirements, simplicity and high reproducibility. ATAC applies Tn*5* transposome-mediated tagmentation which cuts the accessible DNA and simultaneously appends oligonucleotide sequencing adapters to the resulting genomic fragments. In this way, ATAC avoids cumbersome sequencing library preparation as required in alternative approaches using DNases like micrococcal nuclease.

Open promoters of transcribed genes are enriched for histone 3, post-translationally modified by trimethylation at lysine 4, in short H3K4me3, while transcriptionally inactive chromatin is enriched for H3K9me3 [26]. Active enhancers are enriched for H3K27ac and H3K4me1, while H3K27me3 generally marks inactive promoters and enhancers [26]. H3K27me3 and H3K4me3 occur together at bivalent promoters, poised for transcription of developmental genes [27] (see Fig. 2). Mutations in genes which encode histone modifying enzymes or remodeling proteins involved in nucleosome positioning lead to global dysregulation of gene expression and are frequently found in cancer [28]. Missense mutations affecting modification sites in histone genes exert similar global effects, as observed in high-grade childhood brain tumors and in tumors of bone tissue [29,30].

Global profiling of modified histones in the chromatin is based on the enrichment of specific chromatin fragments by chromatin immunoprecipitation followed by NGS (ChIPseq) [31] (see Table 1). In conventional ChIPseq, nuclear proteins are fixed to the DNA by formaldehyde crosslinking followed by ultrasonication of the crosslinked chromatin for fragmentation. Studies integrating gene expression and ChIPseq data have helped to elucidate the underlying mechanism of oncogene upregulation by aberrant enhancer-mediated activation as described, for example, for tumor type-specific TFs in clear cell renal carcinoma [32] and colorectal cancer [33] (for recent review: [34]. Conventional ChIPseq, however, requires up to 10 million cells which might not be available from small tumors. Still working with crosslinked chromatin, ChIPmentation proved an advancement because of its low input requirement due to tagmentation for efficient generation of sequencing libraries [35]. ChIPmentation became recently further streamlined, more efficient and also high-throughput compatible [36]. Other improved alternatives requiring much less cells than conventional ChIPseq are cleavage under targets and release using nuclease (CUT&RUN) [37,38], cleavage under targets and release using tagmentation (CUT&Tag) [39] and antibody-guided chromatin tagmentation (ACT-seq) [40] (see Table 1). These methods work with permeabilized native cells and fusion proteins with high antibody affinity and nuclease or tagmentation activity. Via antibody affinity, nuclease or transposome are guided to the chromatin targets where they cut the DNA into suitable fragments for NGS. It remains to be shown, whether the new methods can also be applied on formalin-fixed biopsies as has been demonstrated with conventional ChIPseq [41]. Since nucleosome positioning and altered histone tail modifications have been reported, for example, in leukemic T cells or chronic lymphocytic leukemia samples [42,43], it can be envisioned that these alterations can be developed into diagnostic/predictive biomarkers.

1.3. Identification of aberrant enhancer-promoter interactions in the cancer chromatin

The three-dimensional (3D) structure of the chromatin contributes to the functional genome organization. Developmental and cell-type specific gene expression programs are orchestrated by the juxtaposition of distant enhancers and promoters enabling the assembly of the transcriptional machinery to initiate transcription [44]. The current model of the functional genomic 3D-architecture describes the genome as folded into higher order looped structures designated chromosome territories and topologically associating domains (TADs) (see Fig. 2) [45]. The concept is largely based on proximity ligation data obtained with the Hi-C approach (see Table 1) in which the 3D-structure of the chromatin is fixed prior to a series of experimental manipulations including DNA ligation such that juxtaposed genomic regulatory elements finally show up in the same sequencing reads [46,47].

Disturbance of the proper genomic 3D-structure can lead to ectopic oncogene activation by aberrant “hijacking” of a distant enhancer. Examples are the activation of the stem cell regulator EVI1 under the control of a distal *GATA2* enhancer in cases of acute myeloid leukemia with an inv(3)/t(3;3) rearrangement [48], activation of the cell cycle regulator CCNE1 under the aberrant control of diverse enhancers by a variety of rearrangements in patients with gastric cancer [49], or the activation of TF NR4A3 in acinic cell carcinomas of the salivary glands due to recurrent [t(4;9)(q13;q31)] rearrangements which juxtapose enhancers from the *SCPP* gene cluster to the *NR4A3* promoter [50]. More systematic approaches using genetic and transcriptomic data for the discovery of structural variant driven proto-oncogene activation has recently been published by the PCAWG Consortium and indicates an unexpected number of such enhancer-hijacking events in the cancer genomes [51,52]. Aberrant ectopic gene activation may also result from neo-enhancers, minor distal sequence alterations which provide new target sites for transactivating proteins. Neo-enhancers with novel binding sites for the transactivator MYB have been observed in cases of T-cell acute lymphoblastic leukemia with oncogenic monoallelic activation of TF TAL1 [53,54]. Circular chromosome conformation capture followed by NGS (4C-seq) enables the identification of aberrant enhancer-promoter interactions in tumor cells [48,55]. Like Hi-C, 4C exploits chromatin crosslinking and proximity ligation (see Table 1). In 4C, the DNA of crosslinked chromatin is digested twice with different restriction enzymes, each time followed by proximity ligation which finally generates circular molecules. PCR with outward-directed primers from the viewpoint, the promoter of the aberrantly activated gene, amplifies the previously unknown interacting region supposed to contain the activating enhancer. Independent confirmation of the enhancer can be achieved by reciprocal 4C with the suspected enhancer as viewpoint. Additional confirmation may be obtained by ChIPseq addressing active enhancer marks H3K27ac and H3K4me1 and by luciferase enhancer reporter assays.

1.4. Single cell epigenomics

Epigenomic analyses are no longer confined to cells in bulk but are now also possible with individual cells to interrogate their DNA methylome [56–58], chromatin accessibility [59,60], chromatin configuration [61,62] and post-translational histone modifications [39,40] at mid- to high-throughput (see Table 1). These methods enable to study cell identity and tissue composition, both in the context of healthy and diseased conditions and, thus, overcome interpretation difficulties introduced by cell type heterogeneity which is common in primary tissue samples. For example, single cell epigenomic variability has been linked to functional heterogeneity in cancer cells [63], and single cell ATAC-seq has been used to identify cell type-specific epigenetic signatures that are associated with type II diabetes [64]. Methods for integrative analysis of multiple information layers, including the mutational status, epigenome profiles and transcription patterns obtained from one and the same cell, are currently being developed [65–67]. From a systems biology perspective, such multi-OMICs approaches further our understanding of the fundamental regulatory mechanisms underlying normal and malignant phenotypes. For example, a multi-OMICs single cell study of gastrulation revealed that epigenetic priming precedes cell-fate decisions in mouse embryogenesis [68]. In cancer research, multi-OMICs single cell studies in combination with pharmacologic or (epi-)genetic perturbations will be crucial to disentangle molecular driver events from collateral biological noise. Presently, multi-OMICs

single cell studies are largely confined to basic research. A recent example used combined single cell DNA methylation and transcriptome analysis together with other OMICS technologies to demonstrate that defects in the DNA methylation machinery alters hematopoietic differentiation by skewed transcriptional priming [69]. As of today, application of multi-OMICs single cell technologies in the clinical setting awaits to be established.

1.5. Epigenetic biomarkers in liquid biopsies

Repeated invasive biopsy resection to study cancer progression can be circumvented by so called liquid biopsies. These are samples taken e.g. from peripheral blood, saliva or urine that contain cell-free DNA (cfDNA) or circulating tumor cells. Epigenetic biomarkers like tumor-enriched non-coding RNA or tumor-specific DNA methylation patterns are attractive non-invasive, diagnostic targets in clinical oncology due to their stability in liquid biopsies [70,71]. Methods of choice for reliable quantification of micro-RNA expression are real-time PCR and deep sequencing. Circulating micro-RNAs are of potential predictive and prognostic value in diverse malignancies including oral, colorectal, endometrial and ovarian cancer [72–75]. MiR-21, for example, is known to silence a variety of tumor suppressor genes and proved predictive and prognostic in several hematological and solid tumor types. cfDNA is usually sparse and highly fragmented. cfDNA concentration in body fluids and its fragmentation and DNA methylation patterns are non-random and reflect characteristics like tumor entity, chromatin accessibility and DNA methylation profile of the original tumor cell [76–78]. As a further example, cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) was applied to non-invasively profile the methylome of patients with primary early-stage pancreatic ductal adenocarcinoma. Comparison with corresponding profiles derived from solid tissue revealed high disease-specific concordance and, hence, suggested that cfDNA methylation patterns can be employed for the non-invasive early detection and classification of solid tumors [78]. A recent technological refinement was the successful methylome profiling of tumor-derived cfDNA after the removal of high molecular weight genomic DNA from normal cells by solid phase reversible immobilization beads followed by low input, whole genome bisulfite sequencing using a commercial kit [77].

1.6. Precision manipulation of the (epi) genome for functional studies

The functional relevance of epigenetic alterations can be analyzed

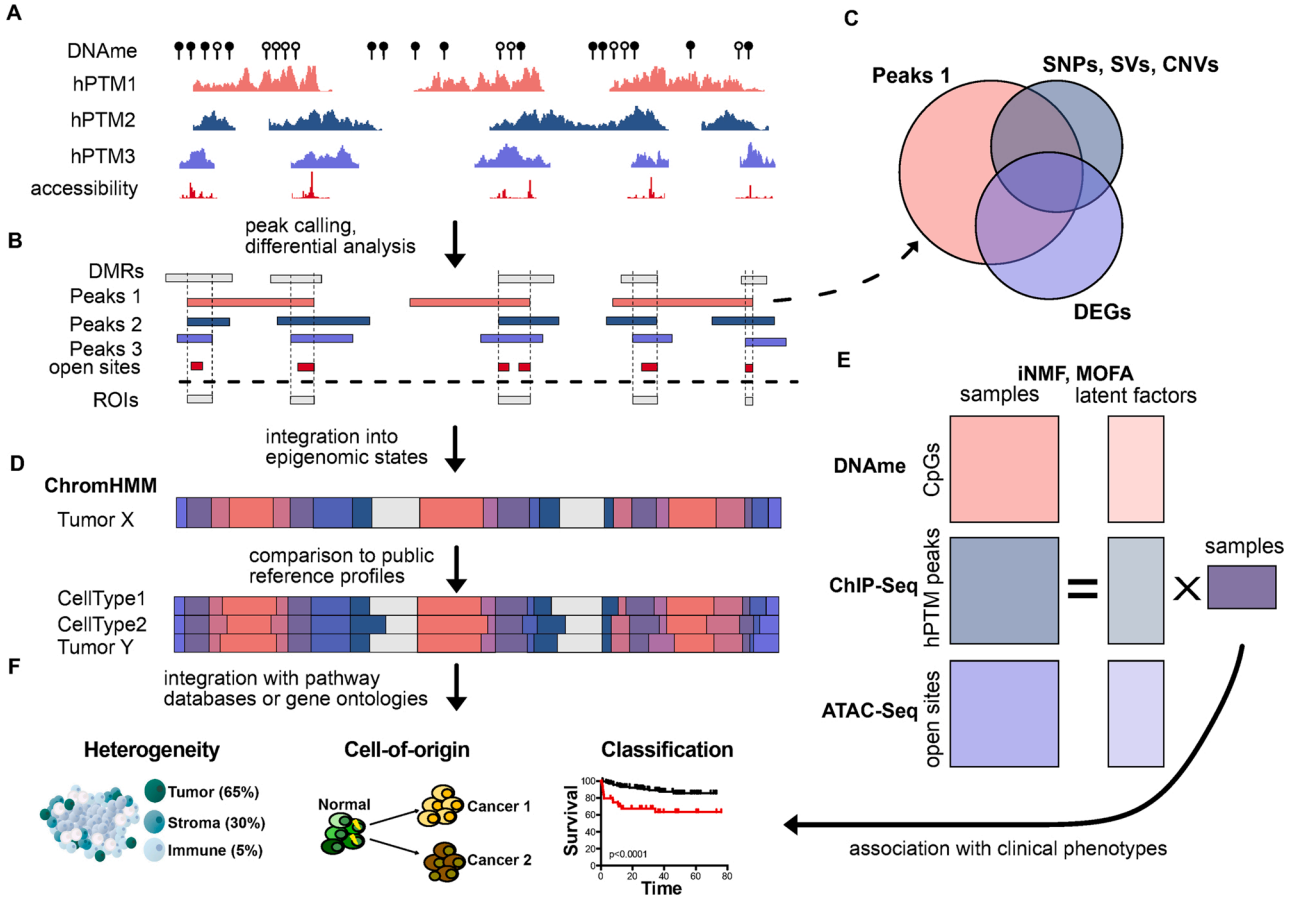

Fig. 3. Integration of epigenomic data. A) Example of epigenomic analysis involving profiling of DNA methylation (DNAme; lollipops), several histone post-translational modifications (hPTM; red, blue and purple tracks), and chromatin accessibility (brown tracks). B) Most basic integration is performed subsequently to calling of hPTM peaks, differential analysis resulting in differential methylation regions (DMRs) and open chromatin sites as simple region overlaps to identify bona fide regions of interest (ROI). C) These regions can be integrated with information from other omics layers, e.g. differentially expressed genes (DEGs), and genetic analysis, including single-nucleotide polymorphisms (SNPs), structural variants (SVs), and copy-number variants (CNVs). D) More advanced strategies involve integration of multiple epigenomic layers into chromatin states, with hidden Markov model-based ChromHMM being by far the most popular approach. ChromHMM state sets for the studied tumor (X) can be uniformly compared to reference profiles of other cell types and tumors (Y). E) Alternatively, data sets with sufficient sample sizes can be summarized into matrices and subject to latent factor analysis methods, e.g. integrative non-negative matrix factorization and MOFA. These methods derive meta-variables (latent factors) integrating information across all the features within each layer as well as contribution of each of these variables to each of the samples. F) High-level information obtained in the integration approaches in combination with other external data can be used for downstream analyses, including tumor heterogeneity, cell-of-origin inference and molecular classification of tumor subtypes.

Table 2
Open problems and possible solutions.

Problem	Solution
Detection of premalignant stages	Population-based long-term studies are needed
Defined tumor- and stage-specific characteristics	Large cohorts are needed, integrated molecular profiling, computational framework
Cell-of-origin identification	Molecular characterization at single cell level or with highly selected cell populations
Non-invasive molecular diagnostics	Analysis of circulating tumor-derived DNA (ctDNA) or cells; enrichment of tumor-specific target molecules
Monitoring of response to treatment and disease recurrence	Sensitive assays for non-invasive diagnostics from ctDNA; knowledge about tumor-specific markers
Personalized therapy	Integrated molecular profiling, defined tumor- and stage-specific molecular characteristics, targeted epigenetic therapies

with genomic manipulation tools like Zinc Finger Nuclease (ZFN), Transcription activator-like effector nuclease (TALEN) or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) [79]. CRISPR/Cas9, in particular, has emerged as a highly efficient manipulation technique [80]. Cas9 nuclease generates double-strand breaks (DSBs) with high precision at target sequences determined by a complementary, target sequence-specific guide RNA. Homology-directed repair of the DSBs in the manipulated cells can be exploited to introduce precise genomic sequence changes [81].

In combination with guide RNA, engineered, nuclease activity-deficient Cas9 (dCas9) specifically binds DNA at target sequences without cleavage. dCas9 tethered to epigenetic editors involved in DNA methylation or demethylation is used to modify the methylation state of regulatory DNA sequences and thereby represses or activates genes [82,83]. Novel modular systems consisting of dCas9 fused to SunTag, a synthetic gene activator, recruit separately transfected epigenetic editors to genomic target sites with higher precision and, thus, reduce off-target DNA modifications frequently observed in earlier systems [84–87]. Similar to its use in targeted DNA modifications, dCas9 has been also exploited in the targeted modification of histones to reactivate silenced target genes, e.g., by increasing promoter-associated H3K4me3 levels [88], or to induce gene silencing, e.g., by de-acetylation of H3K27ac [89]. While these assays most likely will not reach clinical practice in the near future, they will be invaluable tools in helping to understand the molecular consequences of an epigenetic event and subsequently support the researcher in selecting better targets for biomarker development.

1.7. (Epi)genomic data integration

A deep biomolecular characterization of a cancer patient's disease state is a central prerequisite for appropriate treatment and for the development of novel biomarker. To efficiently harness this information from diverse genomic and epigenomic layers such as somatic single nucleotide variants (SNVs), structural variants (SVs), gene expression profiles, DNA methylation patterns and profiles of histone marks, bioinformatic data integration is key (Fig. 3). A simple approach to data integration is to overlap promising regions of interest derived independently from each layer of information. Alternatively, regions from one layer can be used as features to stratify the data of other layers and warrant the interrogation of an additional layer. For instance, intersection of aberrantly expressed genes with differentially methylated regions, SNVs, SVs and sites of histone mark enrichment may warrant targeted chromatin conformation assays like 4C [48]. In general, data derived from a combination of methods like whole genome and transcriptome sequencing, ChIPseq and 4C are integrated to profile cancer-related enhancer-promoter interactions and enable clinically relevant specification of poorly characterized tumors or tumor subclasses [90].

More advanced approaches aim to consolidate heterogeneous epigenetic data by means of integrative statistical models. One such model in widespread use is ChromHMM, a Hidden Markov Model-based algorithm that exploits co-linear histone modification profiles to assign each genomic locus to a distinct chromatin state, e.g., active transcription start site, enhancer or heterochromatic domain (see Fig. 3) [91]. Other methods try to recover hidden (latent) factors that simultaneously act upon several epigenomic layers. As a recent example, Multi-Omics Factor Analysis (MOFA) algorithm fits a model for each layer, with one term for factors shared by all layers, and another term capturing layer-specific modulations of the common factors [92]. Further integrative modelling approaches are based on non-negative matrix factorization [93], kernel methods [94] or similarity network fusion [95] and were extensively reviewed elsewhere [96]. Unobserved epigenetic states as those output by ChromHMM or latent variability factors, derived by methods similar to MOFA, are instrumental both, for mechanistic inference about the biology of respective tumor entities, as well as candidate prioritization in oncological biomarker development and drug design [97,98].

1.8. Summary and outlook

While genetic alterations are widely explored in the context of precision oncology, this is not yet the case for epigenetic modifications, most likely due to the past lack in appropriate technologies. This is now changing and the first examples highlighting the usefulness of DNA methylomes, nucleosome positioning or histone modification patterns for molecular diagnostics have been published for medulloblastomas, ependymomas or chronic lymphocytic leukemia [11,42,99]. Similarly, DNA methylome data can be used to predict response to therapy as shown for juvenile myelomonocytic leukemia [5–7] and for chronic lymphocytic leukemia [12]. Currently the most advanced epigenetic approach to contribute to routine cancer diagnostics in a clinical setting is DNA methylome analysis using EPIC arrays. With targeted gene-specific methods it will also be possible to develop marker panels detecting cancer-specific epigenetic alterations in circulating tumor cells or cell-free DNA isolated from body fluids of tumor patients (Table 2). The workflow for defining novel biomarkers should start with genome-wide profiling for epigenetic alterations, followed by a selection and validation within larger cohorts and finally an improved understanding based on integrative data analysis and molecular evaluations of the consequences of an epigenetic event.

Declaration of Competing Interest

The authors report no declarations of interest.

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