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Review

Compendium of aberrant DNA methylation and histone modifications in cancer

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ABSTRACT

Epigenetics now refers to the study or research field related to DNA methylation and histone modifications. Historically, global DNA hypomethylation was first revealed in 1983, and, after a decade, silencing of a tumor suppressor gene by regional DNA hypermethylation was reported. After the proposal of the histone code in the 2000s, alterations of histone methylation were also identified in cancers. Now, it is established that aberrant epigenetic alterations are involved in cancer development and progression, along with mutations and chromosomal losses. Recent cancer genome analyses have revealed a large number of mutations of epigenetic modifiers, supporting their important roles in cancer pathogenesis. Taking advantage of the reversibility of epigenetic alterations, drugs targeting epigenetic regulators and readers have been developed for restoration of normal pattern of the epigenome, and some have already demonstrated clinical benefits. In addition, DNA methylation of specific marker genes can be used as a biomarker for cancer diagnosis, including risk diagnosis, detection of cancers, and pathophysiological diagnosis. In this paper, we will summarize the major concepts of cancer epigenetics, placing emphasis on history.

Contents

1. Introduction

Epigenetics referred to the study or research field for heritable modifications that regulated gene expression without changes in DNA sequences. The main mechanisms of epigenetic inheritance are recognized to be DNA methylation and histone modifications,

Abbreviations: K, lysine; ac, acetylation; me1, mono-methylation; me2, di-methylation; me3, tri-methylation; DNMT, DNA methyltransferase; FDA, U.S. Food and Drug Administration; POC, proof-of-concept.

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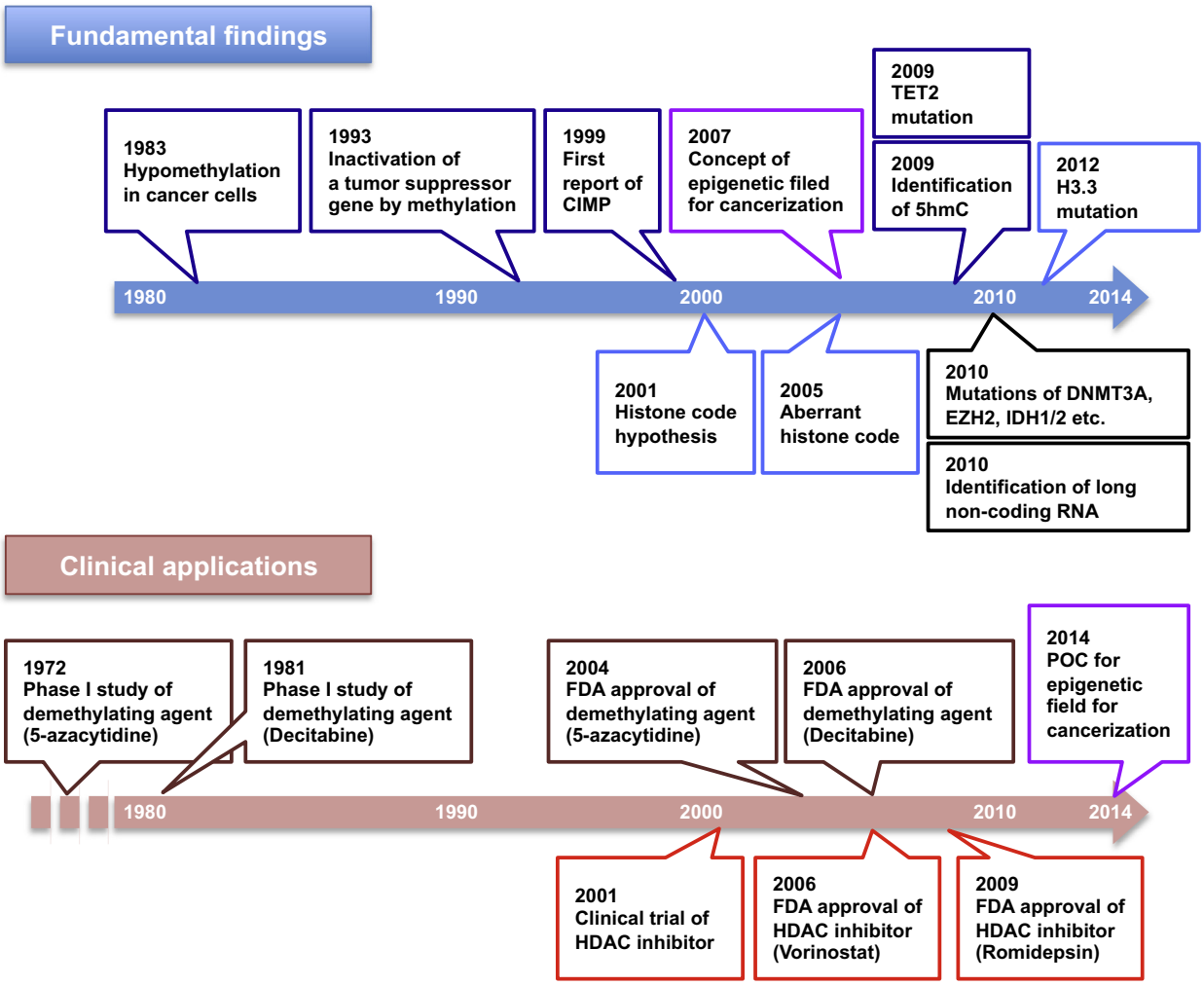

Fig. 1. History of cancer epigenetics. An overview of cancer epigenetics is shown focusing on fundamental findings and clinical applications.

and epigenetics now mainly refers to the study or research field related to DNA methylation and histone modifications. In mammals, epigenetic inheritance is important for pre-implantation development [1], fetal development [2], cell differentiation [3] and tissue differentiation [4]. It is also involved in gametogenesis and cellular reprogramming during the generation of cloned animals and iPS cells [5].

At the same time, aberrant epigenetic modifications (epigenetic alterations) are now considered to be involved in the pathogenesis of several diseases, including pediatric tumors [6]. Especially, aberrant DNA methylation is deeply involved in cancer development and progression because DNA methylation pattern is inherited with a high fidelity in somatic cells [7]. Once aberrant DNA methylation is induced, it is accurately transmitted to daughter cells after cell division. Aberrant DNA methylation is one of the major mechanisms of inactivation of tumor-suppressor genes, along with mutations and chromosomal losses [8].

Historically, the first discovery of epigenetic alterations in cancer goes back to 1983 when global DNA hypomethylation was reported [9]. After a decade, regional DNA hypermethylation was demonstrated to cause silencing of a tumor suppressor gene [10]. The CpG island methylator phenotype (CIMP) was reported first in colorectal tumors in 1999 [11], and thereafter, the presence of CIMP has become known in other types of cancers [12]. As for histone modifications, the impact of histone deacetylase (HDAC) inhibitors (HDACis) on cancer cell proliferation was known in the 1990s [13]. After the proposal of the "histone code" [14], its disturbances have been reported in various types of cancer. Most recently, cancer genome analyses revealed the presence of mutations of epigenetic regulators, including those of *TET* and *IDH* genes [15]. Epigenetic drugs, such as DNA demethylating agents and HDACis, have already become an option for cancer treatment [16].

In this review, we will summarize an overview and trends of cancer epigenetics according to its history (Fig. 1).

1.1. Global hypomethylation in cancer

Global hypomethylation in cancer denotes a decrease in overall content of 5-methylcytosine, and was revealed as the first epigenetic abnormality in cancer by Feinberg and Vogelstein in 1983 [9] (Fig. 1). They analyzed DNA methylation in cancerous and non-cancerous tissues by Southern blotting of DNA digested with methylation-sensitive restriction enzymes, and found a substantial reduction in DNA methylation in cancer tissues. Gama-Sosa and colleagues also investigated the difference in DNA methylation level between cancerous and non-cancerous tissues by a high-performance liquid chromatography, and showed a reduction of 5-methylcytosine content in cancer tissues [17]. Such hypomethylation was also found in pre-malignant adenomas [18,19].

Global hypomethylation involves repetitive sequences, which is observed not only in cancers but also in non-cancerous tissues [20], such as normal mucosae exposed to chronic inflammation

[21]. Global hypomethylation also involves promoter regions of cancer-testis antigen genes, such as *MAGE* and *GAGE* [22,23]. Although several studies investigated DNA hypomethylation of oncogenes [24–27], activation of oncogenes by hypomethylation is still controversial because the regions analyzed in most studies were outside the promoter region that control gene expression and some oncogenes do not have CpG islands (CGIs) in their promoter regions. On the other hand, it has been established that DNA hypomethylation leads to chromosomal instability and tumor development, using DNA methyltransferase 1 (Dnmt1) hypomorphic mouse [11,28,29].

1.2. Regional DNA hypermethylation in cancer

Regional DNA hypermethylation in cancer denotes increased methylation at normally unmethylated CGIs. If a CGI in a gene promoter region is methylated, its downstream gene is consistently inactivated. Therefore, regional hypermethylation in a promoter CGI of a tumor-suppressor gene can inactivate the gene, leading to tumor development and tumor progression [8]. In 1993, inactivation of the *RB* tumor-suppressor gene by DNA hypermethylation of its promoter CGI was reported as the first evidence [10] (Fig. 1). Subsequently, aberrant DNA methylation of other tumor-suppressor genes, such as *CDKN2A* (*p16*) [30], *MLH1* [31] and *CDH1* [32] was also reported as an alternative mode of inactivation to genetic alterations.

As the importance of aberrant DNA methylation was recognized, genome-wide screening techniques to identify aberrantly methylated regions were developed in the late 1990s, including restriction landmark genomic screening (RLGS) [33], methylation-sensitive representational difference analysis (MS-RDA) [34] and methylation-specific arbitrarily-primed PCR (MS-AP-PCR) [35]. In the 2000s, methods using an antibody against methylated cytosine or methyl-CpG-binding domain protein (MBD) [36] were developed. Now, microarray analysis combined with bisulfite treatment, such as Infinium BeadArray for human sample [37], and next-generation sequencing, such as reduced representation bisulfite sequencing (RRBS) [38] and whole-genome bisulfite sequencing [39], are widely used. Such genome-wide analyses revealed that a large number of genes with promoter CGIs (from several hundreds to one thousand) are hypermethylated in cancer [40]. Since most of such genes are not expressed or expressed at very low levels in normal cells, they are considered not as "driver genes", which are causally involved in tumorigenesis, but as "passenger genes", which are methylated by an accompanying phenomenon of carcinogenesis. Until now, a large number of tumor-suppressor genes have been shown to be potentially silenced by aberrant DNA methylation, and aberrant DNA methylation is the one of the major mechanisms that inactivates tumor-suppressor genes.

1.3. The CpG island methylator phenotype

The "CpG island methylator phenotype (CIMP)" is defined as frequent methylation of multiple CGIs, and was first reported in colorectal cancers by Toyota and colleagues in 1999 [11,41] (Fig. 1). The presence of tumors with the CIMP has been reported in other type of cancers, including neuroblastoma [12], glioma [42,43] and gastric cancer [44,45]. Importantly, the CIMP status is uniquely associated with specific clinicopathological characteristics in individual cancer types, indicating that the CIMP provides information for cancer diagnosis and may be utilized to stratify patients for therapeutic opportunities [46].

For instance, the CIMP of colorectal cancers is associated with tumors in elderly patients, in the right-side colon and in female patients [47]. The CIMP of neuroblastoma is strongly associated with poor prognosis in patient cohorts from multiple countries, including Japan, Germany, Italy and Sweden. Moreover, CIMP-positive cases include almost all cases with *MYCN* amplification, a prognostic marker used in clinical practice, and the CIMP status provides prognostic information not provided from *MYCN* amplification [48]. In 2014, the analysis of neuroblastoma CIMP has become commercially available in Japan.

The mechanisms for development of CIMP are one of the most discussed topics in the field of cancer epigenetics [49]. Recent studies by Killian and colleagues and by Turcan and colleagues demonstrated an association between specific genomic alterations and the presence of the CIMP [50,51]. In gastrointestinal stromal tumors, cases with mutation of succinate dehydrogenase (SDH) displayed the CIMP phenotype [51]. In gliomas, IDH1 mutation was shown to be sufficient to establish a glioma with CIMP [50].

1.4. Discovery of 5-hydroxymethylcytosine and the role of TET proteins in cancer

Whether active DNA demethylation is present or not has been a strong debate for a long period as DNA methylation was thought to be a consequence of failure in maintenance methylation after DNA replication. However, finally, a mechanism of active DNA demethylation was proposed by involvement of oxidative demethylation [52]. In 2009, Tahiliani and colleagues showed that the ten-eleven translocation (TET) family proteins could modify 5-methylcytosine to 5-methylhydroxycytosine (5-hmC) by oxidation of the 5-methyl group [53,54]. Now, many investigators trust the presence of active DNA demethylation by TET proteins [55].

TET2, a close relative of TET1, was also reported as an enzyme related with 5-hmC generation. Frequent somatic *TET2* mutations were observed in several hematological cancers, and a low level of 5-hmC was observed in bone marrow samples of patients with *TET2* mutations [56]. As for solid tumors, in 2012, Lian and colleagues demonstrated that loss of 5-hmC was present in melanoma, and that *IDH2* mutations and down-regulation of TET proteins might be the mechanisms of loss of 5-hmC [57]. As genes in which demethylation is important to suppress tumor development and progression, *TIMP2* and *TIMP3* were implicated as TET1-target genes in prostate and breast cancers [58].

1.5. Aberrant histone modifications in cancers

The histone code hypothesis was proposed by Jenuwein and Allis in 2001, to explain how combinations of histone modifications contribute to alterations of chromatin structure and changes of gene expression [14] (Fig. 1). Now, in various cancers, alterations of histone modifications have been reported in both global and gene-specific manners. In 2005, for the first time, Fraga and colleagues reported down-regulation of histone H4K16ac and histone H4K20me3 in colorectal cancer and leukemia [59] (Fig. 1). Importantly, some histone alterations were found to be associated with poor prognosis, such as histone H3K4me1 and H3K9me2, 3 in prostate cancers and histone H3K4me2, H3K9me2 and H3K18ac in pancreatic cancers [60,61]. Gene-specific change of histone marks, such as H3K27me3, H3K9me2 and H3K79me2, inactivated tumor-suppressor genes, resulting in tumor development and progression [62–64].

Before the proposal of the histone code hypothesis, a difference in histone acetylation was reported between cancer and normal tissues [65]. In addition, inhibitors of HDACs were developed and their differentiation induction effect on leukemic cells was noted [66]. The impact of HDACis on cancer cell proliferation was also revealed [13], and now, aberrant histone acetylation in cancer has became a therapeutic target using epigenetic drugs [16].

1.6. Mutations of epigenetic regulators in cancer

Recent genomic analyses using next generation sequencing discovered mutations of epigenetic regulators in cancers [15,67]. In 2009, Abdel-Wahab and colleagues, along with other groups, found frequent mutations in *TET2* in myeloid malignancies, and showed the association between the mutations and decreased overall survival in AML [68,69]. *IDH1* and *IDH2* mutations are also frequently observed in gliomas, and those mutations were shown to lead to loss of their physiological function, conversion of isocitrate into α-ketoglutarate, and to gain of function to produce 2-hydroxyglutarate (2-HG) [70,71]. The metabolite 2-HG competitively inhibited activity of TET1 and TET2, leading to decrease of 5-hmC. 2-HG also inhibited several histone demethylases, such as KDM2A, leading to genome-wide alterations of histone modifications [72].

Mutations of other epigenetic modifiers, including *DNMT3A*, *EZH2* and *SETD2*, have also been identified. When DNMT3A mutation occurs at R882, most frequent in AML, the methyltransferase activity of DNMT3A is decreased [73,74]. It has been reported that in lymphoma, EZH2 mutation at Y641 increased its enzymatic activity, leading to aberrant histone H3K27 methylation [75–77]. An analysis of renal carcinoma showed inactivating mutations of *STED2*, a histone H3K36 methyltransferase, and *KDM5C*, a histone H3K4 demethylase, and *KMD6A*, a histone H3K27 demethylase [78,79]. The investigation of cancer genome accelerated our understanding of cancer epigenome.

1.7. Histone H3.3 mutations in malignant glioma

Specific roles of histone variants in various biological processes were clarified entering the 2010's. In cancers, Schwartzentruber and colleagues demonstrated that 31% of glioblastomas contained somatic mutations in a histone variant, histone H3.3, in 2012 [80]. Mutations in the *H3F3A* gene, K27M and G34R/V, have been identified, leading to amino acid changes in the N-terminal domain of the H3.3 protein. These mutations were mutually exclusive, and the tumors with H3.3 mutation showed distinct profiles of DNA methylation and gene expression [80,81]. Tumors with K27M also displayed a global decrease of H3K27me2 and H3K27me3. These results suggested that the *H3F3A* mutation defined a unique subgroup of gliomas.

1.8. Clinical application as epigenetic cancer therapy

One of the most important characteristics of epigenetic alterations is their reversibility [82]. Drugs targeting epigenetic alterations and regulators have been developed for the purpose of restoration of normal epigenomic pattern, and were shown to have clinical benefits [83]. Two classes of epigenetic drugs, DNMT inhibitors and HDACis, are already in clinical practice.

(a) Development and clinical application of DNA demethylating agents

In 1964, Sorm and colleagues developed 5-azacytidine (azacitidine; 5-Aza; Vidaza®) and 2′-deoxy-5-azacytidine (decitabine; 5-aza-CdR; Dacogen®) as classical cytostatic agents [84]. The possible anti-cancer activity of azacytidine was reported in 1968 using a mouse model of acute leukemia without attention to its ability of DNA demethylation [85]. In 1979, Taylor and Jones revealed its activity to induce cell differentiation *in vitro* and its involvement in the inhibition of DNA methylation [86].

Nevertheless, the first clinical trial of decitabine in patients with acute leukemia, published in 1981, was conducted without much attention to its epigenetic effects [87]. Although a significant reduction in circulating blasts was observed, the dose determined based upon the maximum-tolerant dose induced severe and prolonged myelosuppression, possibly due to the cytotoxic effect of high-dose of decitabine. After implementation of a new regimen focusing on their epigenetic action, namely a low dose and a prolonged exposure, the DNA demethylating drugs exhibited a much better anti-cancer effect [88]. Azacytidine and decitabine were approved in 2004 and 2006, respectively, by the FDA for the treatment of myelodysplastic syndrome (MDS) [89,90].

A new generation of DNMT inhibitors, such as SGI-110, is currently being developed in clinical trials [16]. In addition, a combination with an HDACis or another anti-cancer drug is also being attempted. Indeed, a combination of a DNA demethylating agent and an HDACis was shown to be promising in patients with refractory advanced non-small cell lung cancer [91,92].

(b) Clinical application of HDAC inhibitors

The first clinical trial using HDACis, romidepsin (depsipeptide; Istodax®), reported in 2001, demonstrated to be promising [93]. In 2006, suberoylanilide hydroxamic acid (SAHA; vorinostat; Zolinza®) was approved by the FDA for the treatment of cutaneous T cell lymphoma (CTCL), and romidepsin was also approved for the same indication in 2009. In human, there are 18 HDAC proteins categorized into four classes, class I, IIa, IIb and III. Romidepsin and SAHA target at least four HDACs across multiple classes, showing no strict specificity in their target HDACs. To reduce toxicity associated with such global HDAC inhibition, novel agents are being developed for selective inhibition of specific HDACs. Entinostat and mocetinostat selectively target class I HDACs, and ACY-1215 is a specific inhibitor of HDAC6. Until now, more than 20 different inhibitors are under investigation in clinical trials for hematological and solid tumors [16,94].

(c) Inhibitors targeting other epigenetic modifiers and readers

Other candidates for epigenetic drugs are inhibitors for histone methyltransferases, histone demethylases, and proteins that recognize histone modifications. Histone H3K9 methyltransferase G9a, overexpressed in several type of cancers, could be inhibited by a chemical compound, BIX-01294 [95]. BIX-01294 showed strong anti-growth ability in cancer cell lines with high expression of G9a [96]. Specific inhibitors of EZH2, such as GSK126, have also been developed, and exhibited anti-tumor activity for lymphoma with *EZH2*-activating mutation [97] and rhabdoid tumors with *SMARCB1* mutation [98]. The potent inhibitor of DOT1L, histone H3K79 methyltransferase, could induce selective killing of mixed lineage leukemia cells harboring *MLL* translocation [63]. Bromodomain-containing proteins recognize acetylated histone and function as readers of histone acetylation at super-enhancers [99]. Several inhibitors of such proteins has been developed, and are in phase I trial. For the past few years, the development of epigenetic drugs speeded up across industry and academia, and it is evident that the new epigenetic drugs will be brought into the clinical area.

1.9. Clinical applications as epigenetic cancer diagnosis

DNA methylation of specific marker genes can be used as a biomarker for cancer diagnosis [100]. Generally, cancer diagnosis can be categorized into (a) risk diagnosis, (b) detection of cancers, and (c) pathophysiological diagnosis that estimates cancer responsiveness for therapy and patient prognosis.

(a) Estimation of cancer risk by DNA methylation

Aberrant DNA methylation is observed not only in cancer tissue but also in non-cancerous tissue and pre-cancerous tissue of espe-

cially inflammation-associated cancers, indicating "epigenetic field for cancerization" [101]. Since the epigenetic field reflects the past exposure to carcinogens and/or inflammation, its severity, assessed as aberrant DNA methylation of specific marker genes, can be correlated with cancer risk. As a very convincing prospective cohort study, one report was published in 2014 by Asada and colleagues [102].

(b) DNA methylation markers for cancer detection

For cancer detection markers, samples and sensitivity should be always considered. One of the most promising DNA methylation markers for screening of colorectal cancer is *SEPT9* hypermethylation in blood. In 2011, Warren and colleagues demonstrated that colorectal cancer could be detected in blood-based samples with a sensitivity of 90% and a specificity of 88% [103]. *GSTP1* hypermethylation in urine has been also reported as a promising biomarker to detect prostate cancer with 82% sensitivity and 95% specificity [104].

(c) DNA methylation markers for cancer pathophysiological diagnosis

DNA methylation marker can be used to predict a response to chemotherapy. The promoter hypermethylation of *MGMT*, which codes a DNA repair enzyme, can be used to predict a response of glioma to alkylating agents. In a tumor with unmethylated *MGMT*, its expression can be induced after treatment with an alkylating agent, such as temozolomide, and leads to resistance to alkylating agent. On the other hand, in a tumor with methylated *MGMT*, its expression can never be induced even after the treatment [105].

Response to a therapy can be associated with DNA methylation of not only a single gene but also multiple genes, namely the CIMP. Jover and colleagues demonstrated that colorectal cancer with the CIMP could be resistant to chemotherapy with 5-fluorouracil [106]. In the case of neuroblastoma, the cases with the CIMP have significantly poorer prognosis than those without. Importantly, the predictive ability of the CIMP of neuroblastoma has been shown to be much stronger than that of *MYCN* amplification applied to clinical situation [12,48].

2. Conclusion

Since global hypomethylation was reported in 1983, approximately 30 years have passed. Meanwhile, a large number of findings in cancer epigenetics have been made, and some were brought into cancer therapy and diagnosis. However, there still remain a lot of issues to be solved in the field of cancer epigenetics, such as how mutations of chromatin remodelers are involved in cancer development and how epigenetic alterations are exactly induced by exposure to inflammation. Fortunately, new technologies are now available for epigenetic and genetic analyses, and our understanding of cancer epigenetics will be accelerated. More findings will be brought into cancer diagnosis and therapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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