expert reviews
in molecular medicine

DNA methylation profiling in cancer

Yutaka Kondo[1,*] and Jean-Pierre J. Issa[2]

Aberrant DNA methylation in the genome is found in almost all types of cancer and contributes to malignant transformation by silencing multiple tumour-suppressor genes, sometimes simultaneously. Therefore, deciphering the signature of DNA methylation in each tumour is required to better understand tumour behaviour and might be of benefit for clinical diagnostics and therapy. Recent technologies for high-throughput genome-wide DNA methylation analyses are promising and potent tools for epigenetic profiling. Since epigenetic therapy is now in clinical use or trials for several types of cancers, efficient epigenetic profiling is required. In this review, the current key technologies available to assess genome-wide DNA methylation are introduced and the implications of DNA methylation profiling in human cancers are discussed.

Methylation of a subset of DNA cytosine residues is tightly associated with gene regulation. Generally, DNA methylation occurs in the cytosine-guanine sequence (CpG) in adult cells; however, recent genome-wide methylation analysis revealed that a subset of methylation identified in embryonic stem cells was in a non-CpG context (Refs 1, 2). Approximately 70% of CpGs in the genome are methylated. However, short stretches (up to 2 kb) of CpG-rich regions, which are found in the promoters of around 60% of genes, are generally unmethylated and called 'CpG islands' (CGIs) (Refs 3, 4). CGIs are also found in non-promoter regions: around 50% of CGIs are not coincident with annotated promoters, and their biological function is not well understood (Ref. 5). In contrast to methylation in non-promoter CGIs, methylation in promoter CGIs is closely linked to alterations of chromatin structure and is associated with gene silencing. In mammals, DNA methylation is the most stable epigenetic modification known and is involved in many biological phenomena such as X-chromosome inactivation in females, imprinting, inactivation of repetitive elements and epigenetic reprogramming during mammalian development (Ref. 6).

Epigenetic silencing is described as a 'third pathway' in Knudson's model of tumour-suppressor gene inactivation and can affect gene expression without accompanying genetic changes (Ref. 7); therefore, studies over the past two decades have focused on aberrant DNA methylation in neoplasia. Cumulative analyses revealed that cancer cells generally demonstrate global hypomethylation and regional hypermethylation at selected gene-associated CGIs simultaneously. Aberrant hypermethylation in CGI promoters of critical tumour-suppressor genes has been described,

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Accession information: doi:10.1017/S1462399410001559; Vol. 12; e23; July 2010
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https://doi.org/10.1017/S1462399410001559 Published online by Cambridge University Press

and association between this abnormality and tumour initiation and progression is now widely recognised (Ref. 8).

Recently, two important advances in this field have contributed to its popularity as a research area. First, two nucleoside inhibitors of DNA methylation, 5-azacytidine (AZA) and 5-aza-2′-deoxycytidine (DAC) were established as new treatments for the myelodysplastic syndromes (MDSs), which are heterogeneous haematopoietic disorders characterised by dysplasia of one or more of the myeloid blood cell lineages and an increased risk of acute myeloid leukemia (Refs 9, 10). In addition to these methylation inhibitors, another type of epigenetic drug, histone deacetylase inhibitors, which lead to the accumulation of acetylation in histones, have been found to be effective for treatment of lymphomas (Refs 11, 12). Since numerous studies indicate that all malignancies have both genetic and epigenetic abnormalities, targeting epigenetics is a reasonable and potentially effective approach for cancer treatment. The second advance is in the area of genome-scale DNA methylation analysis technology (Refs 13, 14). Previous DNA methylation profiling, such as two-dimensional gel electrophoresis (restriction landmark genome scanning or RLGS), has been used widely, but is technically challenging (Refs 15, 16). Recent microarray-based technology and more powerful and flexible next-generation sequencing technology have improved the process. These recent developments in DNA methylation analysis techniques can be performed on a genome scale and entire methylomes can be characterised at single-base-pair resolution (Ref. 2).

A link between the above two advances has important implications for cancer diagnostics and treatment. Characterisation of DNA methylation profiling of cancer patients might be of great benefit to determine strategies for optimising epigenetic treatment. In this review, DNA methylation profiling and its clinical implications are discussed.

Significance of DNA methylation in cancer

Cancer cells show loss of global methylation and gain of regional methylation simultaneously. Aberrant distribution of DNA methylation is found in almost all cancer cells. Global hypomethylation may cause genomic instability through impairment of appropriate chromatin structure (Refs 17, 18, 19). In addition, hypomethylation in CGI promoter regions is associated with the derepression of certain cancer-associated genes, such as melanoma antigen genes (MAGEs) (Ref. 20). Loss of imprinting (LOI), leading to pathological biallelic expression of IGF2 (which typically activates the maternally silenced allele), is another well known cause of tumorigenesis (Ref. 21). Although incompletely studied, abnormal gene activation by hypomethylation might mediate the early aberrant clonal expansion of cells and the development of tumours.

Aberrant hypermethylation in promoter CGIs has been comprehensively studied in human cancers, although mechanisms regulating the establishment of de novo DNA methylation remain poorly understood. This DNA methylation in promoter CGIs links to gene silencing in a subset of cancer-related genes (Ref. 22). Many cancers appear to have genes that are silenced by DNA methylation leading to loss of function. Since several molecular pathways are involved in tumour formation, simultaneous silencing of many genes by epigenetic mechanisms could be a strong driving force for transformation (Refs 23, 24). The frequency of DNA methylation in cancerous tissues varies between different genes. In addition, targets of DNA methylation differ depending on the cell of origin of cancers (Ref. 25). One plausible hypothesis to explain selectivity is that DNA methylation occurs in CGIs randomly, with specificity subsequently happening through the process of selection. However, it seems unlikely that all DNA methylation arises in a random fashion, and some methylation might reflect abnormalities in programmes of epigenetic control, which results in methylation patterns that are generated through selective advantage (Ref. 8). Indeed, in genome-wide studies, DNA methylation does not always target tumour-suppressor genes, and many of the hypermethylated genes are already repressed in normal tissues before transformation (Ref. 26). Thus, it also appears likely that CGIs differ in their intrinsic susceptibility to de novo methylation in cancer cells (Ref. 27).

Recently, links between polycomb group protein (PcG)-mediated histone H3K27 trimethylation (H3K27me3) and DNA methylation in cancer were described. The conventional view is that the mark imposed by polycomb repressive complex 2 (PRC2) during

Accession information: doi:10.1017/S1462399410001559; Vol. 12; e23; July 2010
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https://doi.org/10.1017/S1462399410001559 Published online by Cambridge University Press

development predisposes some genes for later de novo methylation (Refs 28, 29, 30). This model appears to be applicable to a set of genes prone to gain DNA methylation during tumorigenesis. However, the presence of H3K27me3 does not always lead to de novo methylation, and H3K27me3 target genes can be methylated in one cancer and unmethylated in another cancer from the same tissue type (Refs 31, 32). Another credible model has been reported recently, in which the existence of stalled RNA polymerase on promoter regions might be a cue to determine the epigenetic fate of promoter DNA methylation (Ref. 33). Although this model is also applicable to a set of genes, several mechanisms that establish de novo DNA methylation appear to be involved in each gene. Further analyses are still required to elucidate how DNA methylation arises in selected CGI promoters.

Significance of the CpG island methylator phenotype

Recent studies in colorectal cancers have indicated the existence of an accumulation of high rates of aberrant promoter hypermethylation in a subset of cases known as CpG island methylator phenotype (CIMP) positive (Refs 34, 35). The mechanisms responsible for causing CIMP tumours is not known; however, CIMP-positive colorectal cancers exhibit distinct genetic and clinical features, including high rates of mutations of *BRAF* and *KRAS*, low rates of *TP53* mutations, a specific histology (mucinous, poorly differentiated), a proximal location and characteristic clinical outcomes (Ref. 9). Furthermore, the existence of different classes of CIMP tumours (e.g. CIMP1 and CIMP2) has been shown recently, raising the possibility that sporadic colorectal cancers arise from distinct parallel pathways, in which the type of genetic and epigenetic instability ultimately explain the characteristic carcinogenesis pathways altered (see below) (Refs 35, 36, 37). Given these distinct clinicopathological features, CIMP-related carcinogenesis might proceed through a specific pathway in which the epigenetic changes that occur in premalignant cells determine subsequent genetic changes, thereby fostering the progression of these clones (Ref. 38).

The criteria for defining CIMP tumours, especially in tumour types other than colorectal cancers, are in development, which has contributed to some of the controversies in this field of study. CIMP was originally proposed when DNA methylation in colorectal cancers was found to have a bimodal distribution, with a group of cancers showing an accumulation of high rates of aberrant promoter hypermethylation and unique biological or clinical features. Published studies on CIMP in human neoplasia (focusing on tumours other than colorectal cancers) are listed in Table 1. Since it is not clear which panel of markers will be the most accurate for identifying CIMP in each tissue type, the frequency and significance of CIMP tumours is still controversial (Refs 39, 40). In addition, the methodology for detection of aberrant DNA methylation in cancer cells is another pragmatic issue. The use of methylation-specific PCR (MSP), a highly sensitive and nonquantitative technique to measure methylation, might sometimes fail to find CIMP in certain tumours (Ref. 39). Indeed, almost every research group that quantitatively studied the methylation status of the genes originally used to define CIMP has confirmed that there is concordant methylation in colon and gastric cancers (Refs 35, 41, 42, 43, 44, 45). Although DNA methylation in gastric cancers does not show as clear a bimodal distribution as found in colorectal cancers, the aggregate data does suggest that a group of CIMP-positive gastric tumours probably arises via a process that is distinct from that of non-CIMP (or less DNA-methylation-dependent) tumours. Better characterisation of this unique molecular subgroup of tumours defined by excessive rates of aberrant promoter methylation will require uniform methodology and marker panels for each type of cancer analysed.

Methodologies for DNA methylation profiling

It is now widely accepted that DNA methylation marks of individual genes or panels defining CIMP are associated with the clinical outcome of cancers. Thus, there is an interest in studying cancers with appropriate panels of markers to develop molecular classification systems relevant to diagnosis and therapy, as has been shown in the examples of colorectal cancer and myelodysplastic syndrome (Refs 10, 35). Recent technological progress now enables us to study DNA methylation profiles comprehensively. The new techniques provide a large amount of information that might be of help to better understand the role of epigenetic changes in the

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https://doi.org/10.1017/S1462399410001559 Published online by Cambridge University Press

https://doi.org/10.1017/S1462399410001559 Published online by Cambridge University Press

Table 1. CpG island methylator phenotypes in human malignancies

Tissue	Marker	CIMP criteria	Frequency of CIMP	Method	Ref.
Colon carcinoma[a]					
	MINT1, MINT2, MINT12, MINT17, MINT25, MINT27, MINT31	≥3	28/49 (57%)	COBRA	34
	MINT1, MINT2, MINT12, MINT17, MINT27, MINT31	≥3	21/41 (50%)	MSP + COBRA	72
	CDKN2A, MLH1, THBS1, MINT1, MINT2, MINT12, MINT25, MINT31	≥3	7/30 (23%)	MSP + COBRA	41
	CDKN2A, MDR1, MINT2	≥2	88/275 (32%)	MSP	73
	CDKN2A, MINT1, MINT2, MINT31, MLH1	≥3	8/47 (17%)	MSP	74
	CDKN2A, MINT1, MINT2, MINT31, MLH1	>3	55/510 (11%)	MSP	75
	CDKN2A, MINT1, MINT2, MINT31, MLH1	≥2	256/864 (29.6%)	MSP	76
	CDKN2A, MINT1, MINT2, MINT31, MLH1	≥3	21/208 (10.1%)	MSP	77
	CDKN2A, MDR1, MINT2	≥2	67/206 (32.5%)	MSP	63
	CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1	≥3	33/187 (18%)	MethyLight	78
	CACNA1G, CDKN2A, CRABP1, MLH1, NEUROG1	≥4	78/460 (17%)	MethyLight	79
	CDKN2A, p14ARF, *TIMP3, MGMT, RARB*	≥3	42/161 (26%)	MethyLight	80
	CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3, SOCS1	CIMP-low: 1–5	353/920 (38%)	MethyLight	81
		CIMP-high: ≥6	136/920 (15%)		
	CDKN2A, MINT1, MINT2, MINT31, MLH1	≥2	26/97 (28%)	Pyrosequencing	42
	CDKN2A, MINT1, MINT2, MINT12, MINT31	≥2	49/97 (51%)	MSP, COBRA, pyrosequencing	35
Colorectal cancer liver metastasis					
	CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1	≥3	3/30 (10%)	MethyLight	82
Colon adenoma					
	CDKN2A, MINT1, MINT2, MINT12, MINT31	≥2	46/205 (22%)	MSP	83
	CDKN2A, MINT1, MINT2, MINT31, MLH1	CIMP-low: 1	Serrated, 4/22 (18%)	MSP	84
		CIMP-high: ≥2	Serrated, 15/22 (68%)		
		CIMP-low: 1	Tubular, 6/34 (18%)		
		CIMP-high: ≥2	Tubular, 13/34 (38%)		

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Table 1. CpG island methylator phenotypes in human malignancies *(continued)*

Tissue	Marker	CIMP criteria	Frequency of CIMP	Method	Ref.
	CDKN2A, MINT2, MINT31	CIMP-low: 1 CIMP-high: ≥2	24/76 (32%) 13/76 (25%)	MSP	85
	CDKN2A, MLH1, MGMT, MINT1, MINT2	CIMP-high: ≥2	26/76 (34.2%)	MSP	86
	MINT1, MINT2, MINT12, MINT31	CIMP-low: 1 or 2 CIMP-high: ≥3	23/57 (40%) 25/57 (44%)	COBRA	87
Colon hyperplastic polyp					
	CDKN2A, MINT1, MINT2, MINT31, MLH1	CIMP-low: 1 CIMP-high: ≥2	14/102 (14%) 43/102 (43%)	MSP	88
Gastric cancer					
	MINT1, MINT2, MINT12, MINT25, MINT31	≥3	23/56 (41%)	MSP + COBRA	43
	CDKN2A, MLH1 THBS1, MINT1, MINT2, MINT12, MINT25, MINT31	≥3	10/32 (31%)	MSP + COBRA	41
	MINT1, MINT2, MINT12, MINT25, MINT31	CIMP-I: 2 CIMP-H: ≥3	15/79 (19%) 29/79 (37%)	MSP + COBRA	44
	MINT1, MINT2, MINT12, MINT25, MINT31	≥3	42/103 (41%)	MSP	89
	CDKN2A, MLH1, THBS1, MINT1, MINT2, MINT12, MINT31	≥3	25/105 (24%)	MSP + COBRA	90
	CDKN2A, MLH1, MINT1, MINT2, MINT25, MINT31	CIMP-low: 1 or 2 CIMP-high: ≥3	46/83 (56%) 26/83 (31%)	MSP	91
	MINT1, MINT2, MINT12, MINT25, MINT31	CIMP-low: 1–3 CIMP-high: ≥4	19/78 (24%) 39/78 (50%)	COBRA	45
Gastric MALT					
	CDKN2B, CDKN2A, TP73, MLH1, DAPK, MINT1, MINT2, MINT31	≥3	13/21 (62%)	MSP	92
	CDKN2B, CDKN2A, DAPK, MLH1, MINT1, MINT2, MINT31	≥3	8/13 (62%)	MSP	93
Pancreatic cancer					
	RARb, CDKN2A, CACNA1G, TIMP-3, THBS1, MLH1, MINT1, MINT31	CIMP-I: 2–3	4/36 (11%)	MSP	94
		CIMP+: ≥4	5/36 (14%)		
Cholangiocarcinoma					
	MINT1, MINT12, MINT25, MINT31, MINT32	≥3	18/79 (22.8%)	MSP	95
HCC					
	CDKN2A, CACNA1G, COX2, ER, MINT1, MINT2, MINT27, MINT31	CIMP-I: 1 or 2 CIMP+: ≥3	36/85 (42%) 32/85 (38%)	MSP + COBRA	96
	CDKN1A, CDKN2B, CDKN2A, p53, RB, P27, WTI, E2F-1, p300	≥5	74/120 (61.7%)	MSP	97

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Table 1. CpG island methylator phenotypes in human malignancies *(continued)*

Tissue	Marker	CIMP criteria	Frequency of CIMP	Method	Ref.
Cirrhosis					
	CDKN1A, CDKN2B, CDKN2A, p53, RB, P27, WTI, E2F-1, p300	≥5	18/120 (15%)	MSP	97
	CDKN2A, p14ARF, MLH1, MGMT, MINT1, MINT25, MINT27, MINT31	CIMP-I: 1 or 2	7/12 (58.3%)	MSP + COBRA	98
		CIMP+: ≥3	1/18 (5.6%)		
Lung cancer					
	CDH13, CRBP1	≥1	26/79 (33%)	MSP	99
	VHL, RAR-B, MLH1, SEMA3B, RASSF1A, BLU, FHIT, hOGG1	≥4	34/60 (56.7%)	MSP	100
	RPRM, CDKN2A, SPARC, GREM, IL-12Rb2, RUNX3, SFRP1, SFRP2, SFRP5, Wif-1, Dkk3, APC	≥5	99/229 (43%)	MSP	101
Neuroblastoma					
	17 CGIs of *PCDHB* family	Methylation level ≥40%	67/140 (48%)	MSP	102
Leukaemia					
	ADAMTS1, ADAMTS5, APAF1, ASPP1, CDH1, CDH13, DAPK, DIABLO, DKK3, LATS1, LATS2, NES1, p14ARF, CDKN2B, CDKN2A, CDKN1C, TP73, PARK2, PTEN, SFRP1, SFRP2, SFRP4, SFRP5, SHP1, SYK, TMS1, WIF1	≥3	38/50 (76%)	MSP	103
Neuroendocrine tumours					
	MLH1, APC, CDKN2A, p14ARF, TIMP3, RUNX3, HIC1, PTEN, RARb2, MINT1, MINT2, MINT31	≥3	31/72 (43%)	MSP	104
Pheochromocytoma					
	CDH1, DCR2, RARB, TP73, APC, DAPK1, PTEN, CDKN2A, p14ARF, RASSF1A, NORE1A	≥3	7/53 (13%)	Pyrosequencing	105
Retinoblastoma					
	CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1	≥3	0/19 (0%)	Q-MSP	106
Uveal melanoma					
	CACNA1G, IGF2, NEUROG1, RUNX3, SOCS1	≥3	0/20 (0%)	Q-MSP	107

[a]More papers than listed here have studied CIMP in colorectal cancers. Representative examples are listed.
Abbreviations: CGI, CpG island; CIMP, CpG island methylator phenotype; COBRA, combined bisulfite restriction analysis; MALT, mucosa-associated lymphoid tissue; MSP, methylation-specific PCR; Q-MSP, quantitative-methylation-specific PCR.

Accession information: doi:10.1017/S1462399410001559; Vol. 12; e23; July 2010
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pathogenesis of human cancers. Using these techniques, one can translate the data on aberrant DNA methylation in cancer into clinical practice, which could result in a better outcome for patients.

Some of the available techniques and their features are described in Figure 1. Methylated cytosine is indistinguishable from unmethylated cytosine by standard sequencing approaches. Therefore, before analysis by high-throughput applications, enrichment for methylated DNA or sodium bisulfate conversion are generally used to overcome this problem. Two methods are widely used to enrich for methylated DNA: affinity purification and restriction-enzyme digestion. There are two commonly used affinity purification methods – methylated DNA immunoprecipitation (MeDIP) and the methylated-CpG island recovery assay (MIRA) (Refs 46, 47, 48). In MeDIP, an antibody specific for methylated cytosine is used to immunocapture methylated genomic fragments (Refs 46, 47). The resulting enriched fraction can be subjected to standard DNA detection; thus, MeDIP can be combined with microarrays or large-scale sequencing analysis. However, to apply this technique to a large number of samples, the large amount of starting DNA and the high cost of the antibody can be a problem. MIRA is based on the high affinity of the complex of methyl-binding domain protein 2b (MBD2b) and MBD3L1 to methylated DNA (Ref. 48). Methylated DNA fragments can be captured and purified on an MBD column, and then subjected to analysis by microarrays or sequencing. Differential restriction enzyme digestion is the classical method for detecting methylated DNA fragments. The most widely used methylation-sensitive restriction enzymes are *Hpa*II and *Sma*I, which have methylation-insensitive isoschizomers (*Msp*I for *Hpa*II) or neoschizomers (*Xma*I for *Sma*I) (Refs 49, 50, 51, 52, 53). A combination of these enzymes followed by ligation-mediated PCR enables the enrichment of methylated DNA fragments.

Sodium bisulfate treatment of DNA is now a fundamental method for DNA methylation analysis (Ref. 54). This treatment causes deamination of unmethylated cytosine to uracil, while leaving methylated cytosine intact. After PCR amplification, which converts uracil to thymine, the cytosine-to-thymine signal ratio is directly proportional to the level of DNA methylation at any given position. This method has been combined with pyrosequencing analysis (Ref. 55), restriction-enzyme digestion (combined bisulfite restriction analysis or COBRA) (Ref. 56) or amplified with specific primers (MSP) (Ref. 57) and is a very useful option to analyse the DNA methylation status in selected genes. However, this method is difficult to combine with genome-wide approaches. After bisulfate conversion, cytosines at non-CpG sites are all converted to thymine, which results in a reduction of sequence complexity. However, recently, an array approach (Illumina BeadsArray technology) was described that appears to overcome this problem (Ref. 58). To adapt bisulfite-converted samples to next-generation sequencing platforms, more efficient approaches have been developed that use microarray capture (Ref. 59), size-fractionation after restriction enzyme digestion (reduced representation bisulfite sequencing or RRBS) (Ref. 60) or other types of sequence capture (Refs 61, 62). These techniques effectively enable the adaptation of the bisulfite-converted DNA to the next-generation sequencing platforms. Bioinformatics and biostatistics are also important challenges when bisulfite-converted DNA is sequenced by next-generation sequences, because sequence-based analyses produce more comprehensive massive data sets, which require a practical alignment to a reference genome. Once sequencing costs come down and convenient bioinformatic analyses are easily accessible, it will also be possible to routinely analyse the whole genome by shotgun bisulfite sequencing.

Analysis of DNA enriched for methylated regions can be analysed by microarrays or by next-generation sequencing platforms. Microarray analysis has been successfully used to study large genomic regions. However, this technique suffers from limitations such as cross-hybridisation and lower resolution (analysed regions are limited by the probes) compared with next-generation sequencing. However, microarray analysis is now relatively economical and applicable to a large number of samples, especially because multiplexed array platforms are available. In addition, any probe can be easily selected and mounted on the multiplex platform, which allows the study of different regions [focusing on 5′-untranslated regions (UTRs), 3′ UTR, coding exons etc.]. The next-

Accession information: doi:10.1017/S1462399410001559; Vol. 12; e23; July 2010
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https://doi.org/10.1017/S1462399410001559 Published online by Cambridge University Press

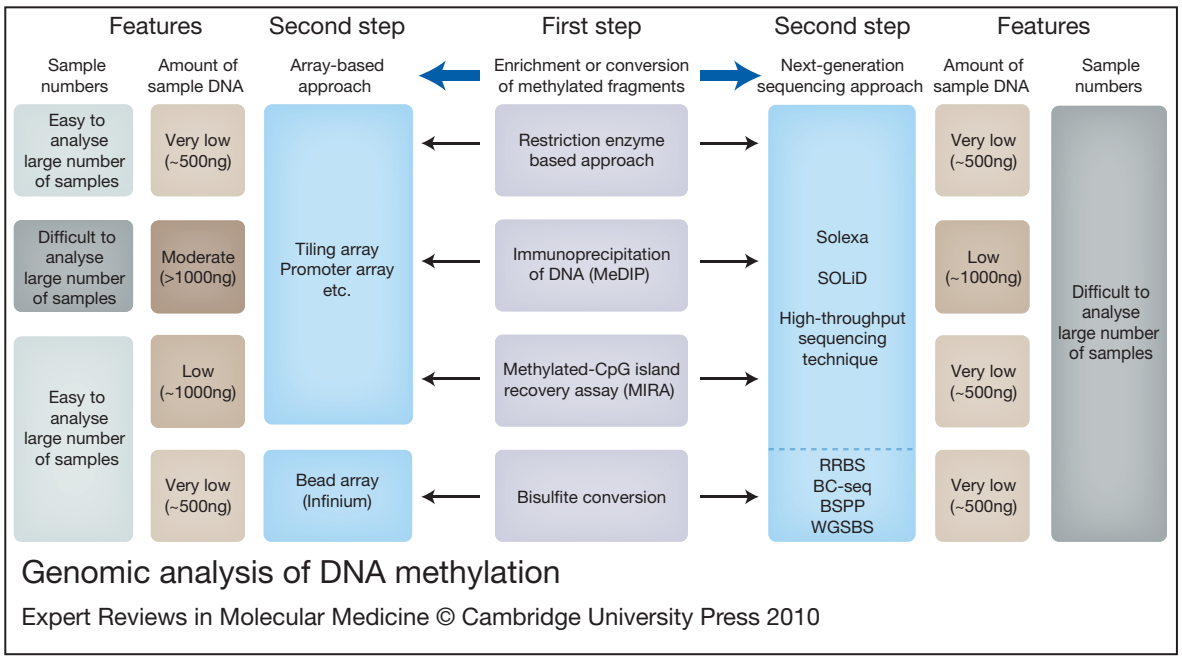


Figure 1. Genomic analysis of DNA methylation. The enrichment of methylated DNA or bisulfite-converted DNA is followed by array-based analysis or high-throughput sequencing analysis. The former is easy to study in a large number of samples owing to the multiplex array platform, whereas the latter has difficulties dealing with large numbers of samples. Since bisulfite-converted DNA has a reduced sequence complexity, several approaches are needed to apply this technique to high-throughput sequencing analysis, such as RRBS (reduced representation bisulfite sequencing), BC-seq (bisulfite conversion followed by capture and sequencing), BSPP (bisulfite padlock probe) and WGSBS (whole-genome shotgun bisulfite sequencing).

generation sequencing platform is a more powerful technique to analyse DNA methylation genome-wide and can overcome some of the weaknesses of a microarray-based approach, with current limitations being cost and throughput. Because of its accuracy and abundant information recovered, genome-wide DNA methylation analysis will shift from microarrays to next-generation sequencing in the near future.

Clinical implications of DNA methylation profiling for human malignancies

DNA methylation in particular genes is associated with clinical outcome. For example, methylation of *MLH1*, which encodes a mismatch repair protein whose loss leads to microsatellite instability (MSI), is associated with a better prognosis in colorectal cancer (Refs 35, 63). DNA methylation in the O6-methylguanine DNA methyltransferase gene (*MGMT*), which encodes a DNA-repair enzyme, is associated with sensitivity to alkylating agents in brain tumours (Ref. 64). Aberrant DNA methylation of the checkpoint with forkhead-associated and ring finger (*CHFR*), a mitotic checkpoint gene, is associated with sensitivity to microtubule inhibitors in colorectal and gastric cancers (Refs 65, 66). Methylation of death-associated protein kinase (*DAPK*) is associated with early recurrence of bladder cancer (Ref. 67). In addition to the relationship between DNA methylation in single genes and clinical outcome, recent integrated analysis between genetic and epigenetic changes in colorectal cancer demonstrated that these cancers can be divided into three molecularly distinct subclasses: (1) CIMP1: *MLH1* and *BRAF* mutations and rare *KRAS* and *TP53* mutations; (2) CIMP2: *KRAS* mutations and rare *MLH1*, *BRAF* or *TP53* mutations; (3) CIMP-negative: high rate of *TP53* mutations and lower rates of MSI or mutations of *BRAF* or *KRAS* (Ref. 35). CIMP1 colorectal cancer tends to have a good prognosis, whereas CIMP2 colorectal cancer is resistant to chemotherapy (5-FU or cetuximab) and generally has poor prognosis (Ref. 40). These findings imply that understanding the

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genetic and epigenetic alterations that promote human cancers and the identification of appropriate biomarkers are prerequisite for successful cancer therapy.

Very recently, genome-wide DNA methylation profiling using a restriction-enzyme-based approach (*Hpa*II tiny fragment enrichment by ligation-mediated PCR or HELP) reveals the existence of distinct DNA methylation patterns in acute myeloid leukaemia (AML) and identifies new, biologically and clinically relevant defined AML subgroups (Ref. 68). The study identified a 15-gene DNA methylation classification that is capable of predicting overall survival in AML patients, even in patients for whom clinical biomarkers are not currently available. Similar studies using genome-wide DNA methylation analysis have been done in hepatocellular carcinoma, colorectal cancer and mesothelioma (Refs 42, 52, 69, 70).

Since genetic alterations are generally irreversible, but epigenetic alterations underlying tumorigenesis can be modulated by drug treatment, typing or profiling of DNA methylation in each cancer might be useful for therapeutic benefit. The DNA methylation inhibitors AZA and DAC, are now used for treatment of myelodysplastic syndrome. It is important to recognise that although the inhibition of DNA methylation is the molecular effect of these drugs, their downstream effects on neoplastic behaviour are nonspecific (Ref. 71). This nonspecificity makes the prediction of clinical outcome difficult. Thus, the responses might be predominantly apoptosis mediated and rapid in some patients, whereas in others, a slow differentiation effect is prominent. To understand the effects of DNA methylation inhibitors more precisely, genome-wide DNA methylation profiling before and after treatment may be useful. This approach could identify novel markers for successful epigenetic therapy.

Conclusions

The contribution of aberrant DNA methylation to carcinogenesis was first reported almost two decades ago. Early cancer research focused on the fact that neoplastic cells have aberrant gene expression via DNA methylation, which results in deregulation of functional pathways. The focus is now shifting to understand aberrant genome-wide DNA methylation in human malignancies, because current knowledge indicates that all malignancies have a mixture of genetic and epigenetic defects. Epigenetic dysregulation of gene expression is theoretically reversible by small molecules. The epigenetic potential of such DNA methylation inhibitors might be limited by other epigenetic mechanisms, such as histone modifications, which are also worth exploring as therapeutic targets. Deciphering epigenetic information, including DNA methylation, in each cancer, and applying this information to the selection of appropriate therapy, including epigenetic therapy, is likely to transform cancer treatment in the future.

Acknowledgements

We appreciate the peer-reviewers' thoughtful comments on this review. Research in Y.K.'s laboratory is supported by grants from a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan, and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

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Further reading, resources and contacts

Publications

Issa, J.P. and Kantarjian, H.M. (2009) Targeting DNA methylation. Clinical Cancer Research 15, 3938-3946
This review provides a detailed description of DNA demethylation as a cancer treatment.

Laird, P.W. (2010) Principles and challenges of genome-wide DNA methylation analysis. Nature Reviews Genetics 11, 191-203
This review describes comprehensively the recent approaches and their relative merits for genome-wide DNA methylation analysis.

Features associated with this article

Figure
Figure 1. Genomic analysis of DNA methylation.

Table
Table 1. CpG island methylator phenotypes in human malignancies.

Accession information: doi:10.1017/S1462399410001559; Vol. 12; e23; July 2010
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https://doi.org/10.1017/S1462399410001559 Published online by Cambridge University Press

Citation details for this article

Yutaka Kondo and Jean-Pierre J. Issa (2010) DNA methylation profiling in cancer. Expert Rev. Mol. Med. Vol. 12, e23, July 2010, doi:10.1017/S1462399410001559

Accession information: doi:10.1017/S1462399410001559; Vol. 12; e23; July 2010
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