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Deciphering the cancer imprintome

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Abstract

Genetic events alone cannot explain the entire process of carcinogenesis. It is estimated that there are more epigenetic alterations in cancer than DNA mutations, and disiphering driver and secondary events is essential to understand early processes of tumorigenesis. Epigenetic modifications control gene activity, governing whether a gene is transcribed or silent. In cancer, global patterns of two epigenetic marks, histone modifications and DNA methylation, are known to be extensively deregulated. Tumour cells are also characterized by loss-of-imprinting, a key epigenetic developmental mechanism. Genomic imprinting is the parent-of-origin, monoallelic expression of genes and is controlled by differentially DNA-methylated regions and allelic-histone modifications. With specific emphasis on imprinted loci this review will discuss alterations in DNA methylation and histone modifications in cancer. The recent advances in technology that might facilitate the identification and characterization of the epigenetic profiles of cancer will also be described.

Keywords: *DNA methylation; imprinting; cancer; histone modifications; epigenetics*

INTRODUCTION

Cancer is as much an epigenetic disease as a genetic one. In addition to genetic aberrations, a series of epigenetic disruptions occur within a cell, favouring uncontrolled growth and allowing for the transformation to a cancer cell. The term 'epigenetic' describes a heritable but reversible change to the structure of DNA, without any change in the sequence. Epigenetic mechanisms control gene expression whereby the interplay between DNA packaging elements ensures a balance between transcriptional activation and repression. This dynamic regulation involves DNA methylation, nucleosome shuttling and histone variants, along with a series of deacetylation, methylations and other modifications at key histone amino-acid residues. All of these epigenetic features are associated with imprinted genes. These allele-specific transcripts, of which there are around 60 in the human genome (http://igc.otago.ac.nz/home.html) [1], constitute a particularly interesting example of epigenetic regulation, since in an individual cell there are active and repressed alleles of the same gene.

HISTONE COVALENT MODIFICATION

Eukaryotic DNA is packaged into chromatin consisting of nucleosomes formed by wrapping 146 base pairs of DNA around an octamer of four core histones (H2A, H2B, H3 and H4). Histone proteins are highly conserved throughout evolution. Histones, particularly their protruding N-terminal tails, are subject to a large number of post-translational modifications [2]. Chromatin state is dynamic and can be divided into two types, silent heterochromatin and active euchromatin. Each of these states is associated with distinct sets of histone modifications. Acetylation, methylation, phosphorylation and ubiquitination of histone tails have been implicated in active transcription, whereas methylation, ubiquitination, sumoylation, deimination and proline isomerization are associated with transcriptional silence. Additional complexity comes from the fact that methylation at lysines can be in the form of either mono-, di- or trimethylation, and mono- and di (asymmetric or symmetric) for arginines. These histone methylation marks at lysine (K) and

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arginine (R) residues are relatively stable and can carry epigenetic information though cell division.

Euchromatin is an open chromatin state, permissive for transcription and the chromatin within these regions has high levels of acetylation and methylation at Lysine 4 of histone H3 (H3K4) [3, 4]. In contrast, heterochromatin is a highly compact chromatin structure also known as the '30 nm fibre', where the DNA is largely inaccessible. It is found throughout the genome both constitutively, at telomeres and centromeres, and facultatively, in coding regions. In mammals, a silent heterochromatic state is associated with DNA methylation, hypoacetylation and high levels of methylation of H3K9 and H4K20 [5]. In addition, the compact structure of the 30 nm heterochromatin fibre is associated with H1 linker histones that arrange themselves between the core histone octamers [6].

Histone modifications are a particularly interesting mechanism to control transcription as the process is extremely dynamic, and sometimes reversible. This is particularly key for cellular differentiation, where epigenetic profiles can be finely tuned to produce all the tissues of the body from only a few embryonic stem cells. In the last five years numerous histone demethylases have been identified that target specific histone residues. For example LSD1 and AOF1/KDM1B are known to demethylate H3K4, and the JmjC-domain-containing proteins target both H3K9 and H3K36, which in turn can delocalize other epigenetic regulators such as HP1 [7]. Lastly, UTX and JMJD3 have recently been identified as H3K27 demethylases [8].

INTERACTIONS BETWEEN HISTONE MODIFICATIONS AND DNA METHYLATION

Histone methylation is thought to be a long-standing and stable modification. Most known sites of histone lysine methylation occur on H3 and H4. The repressive nature of histone methylation on certain residues is partly due to the association with DNA methylation, as many proteins involved in DNA methylation interact with histone modifying enzymes. These processes indicate a convergence of the two pathways in cooperative gene silencing. Genes that are silenced by DNA hypermethylation are not associated with active histone marks, such as H3ac and H3K4me, whereas almost every repressive histone mark, including H3K9me, H3K27me H4K20me are enriched where DNA is hypermethylated (Figure 1). This is because hypermethylated regions are enriched for the catalytic histone methyltransferase (HMTs) enzymes G9a, Suv39h and EZH2 that are responsible for the methylation of H3K9me2, H3K9me3 and H3K27me3, respectively [9]. Furthermore, all three DNA methyltransferases (DNMTs) are known to interact with EZH2 to DNA-methylate EZH2-binding promoters [10]. This observation has important mechanistic implications for determining which genes become aberrantly hypermethylated in cancer. It has recently been reported that many genes involved in early differentiation are associated with the co-enrichment of both H3K27me3 and H3K4me in embryonic stem cells [3]. This combination of histone modifications has been termed bivalent chromatin. This ES cell epigenotpe has been reported to be especially susceptible to cancer associated hypermethylation later in life [11], presumably due to the pre-existing H3K27me3 attracting the DNA methylation machinery.

GENOME-WIDE HYPOMETHYLATION AND PROMOTER HYPERMETHYLATION IS THE HALLMARK OF THE CANCER EPIGENOME

In mammalian cells, methylation occurs by a covalent modification of DNA in which a methyl group is transferred from S-adenosylmethionine to the C-5 position of cytosine by a family of cytosine methyltransferases (DNMTs). Generally, only cytosine bases that are located within a CpG dinucleotide are methylated, but it is now evident that non-CpG methylation exists in mammals [12]. CpG dinucleotides are under-represented in the genome due to the process of spontaneous deamination that converts methylated cytosines to thymidines. However, there are regions of high CpG content known as CpG islands that are evolutionarily protected from the process. Most CpG islands map proximally to the transcriptional start site of genes, and are generally unmethylated. DNA methylation is regulated by a family of DNMTs that comprise of DNMT1, DNMT3A and DNMT3B. Both DNMT3A and DNMT3B are required for *de novo* methyltransferase activity that sets up DNA-methylation patterns early in development [13]. DNMT1 is the most abundant

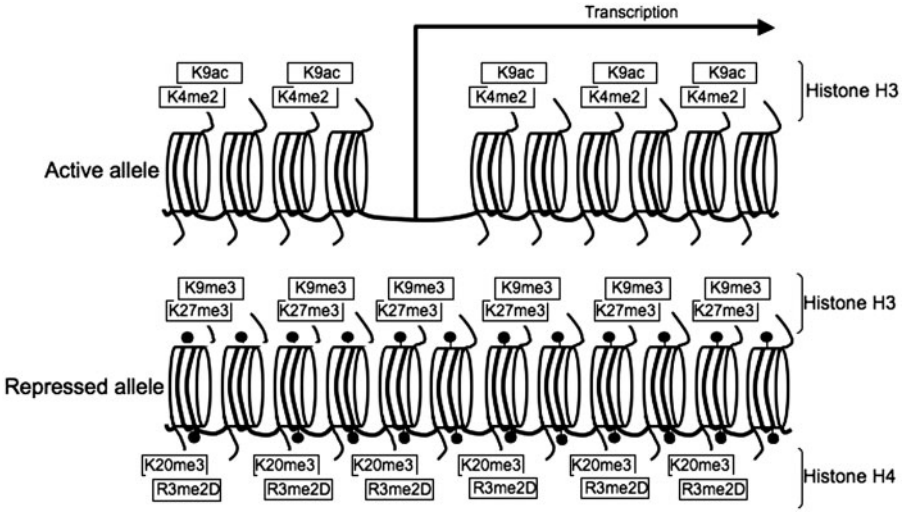

Figure 1: A schematic representation of the chromatin structure of imprinted-DMRs. The active allele is hyperacetylated and is enriched for lysine 4 di- and trimethylation of histone H3. On the methylated allele (filled circle), there is trimethylation of lysine 9 and 20 on histones H3 and H4, respectively, accompanied by H4 arginine 3 methylation. Some, but not all DMRs are associated with trimethylation of lysine 27 of histone H3.

DNA methyltransferase in somatic cells, and responsible for copying DNA-methylation patterns to the daughter strands during DNA replication [14].

Compared to normal cells, human cancers cells show a drastic change in DNA-methylation status, generally exhibiting global DNA hypomethylation accompanied by region-specific hypermethylation. DNA hypomethylation in cancer causes chromatin decondensation, activation of endogenous retroviral elements and chromosomal instability that can result in chromosomal rearrangements. DNA hypermethylation of gene promoters results in silencing of specific genes, including tumour suppressors. This aberrant disruption of DNA methylation in cancer is partly due to inappropriate expression of the DNMT enzymes. Over-expression of DNMT1 leads to hypermethylation [15, 16], while the global hypomethylation correlates with the expression of an abnormal DNMT3B isoform, DNMT3B4, which lacks the conserved methyltransferase motifs [17].

Extensive global hypomethylation allows for the aberrant re-expression of repeat elements, many of which are integrated latent retroviral sequences. The reduction in DNA methylation in the promoter regions of the *HERV-K*, *HERV-W* and *LINE-1* retro-elements in kidney and ovarian cancer results in aberrant expression of these repeats [18, 19]. In additional, recurrent unbalanced chromosomal translocations with breakpoints in hypomethylated pericentric DNA have been reported in many cancer types [20]. Gene specific hypomethylation is a relatively late event, occurring in the final stages of tumour development. Hypomethylation of genes is usually accompanied by reactivation of transcription, either of oncogenes such as *c-Myc* and *c-Ha-RAs*, or tissue- and germline-specific genes such as *MAGE-A1* and *MAGE-A3* [21, 22].

Promoter hypermethylation leading to the transcriptional silencing of putative tumour suppressor genes is another well-characterized epigenetic phenomenon in human tumours. Aberrant promoter hypermethylation is associated with loss of gene expression, which can provide a selective advantage for transformation, similar to that observed for classical genetically mutated tumour suppressor genes. DNA-hypermethylation is known to silence many genes that regulate a number of key cellular processes including the cell cycle (*CDKN2A/p16-INK4*, *CDKN2B/p15/INK4B*, *CDKN1C/p57KIP2*, *CCND2*, *RB1*), DNA repair (*MGMT*, *BRCA1*, *MLH1*), apoptosis (*DAPK*, *TP73*), invasion and angiogenesis (*TFPI2*), metastasis (*CDH1*, *CDH13*), RAS (*RASSF1*) and WNT signalling (*APC*, *DKK1*). The hypermethylation observed at some of these genes may be common to tumours of different origins, while others might be limited to a specific cancer type. Many of the genes implicated in sporadic and familial cancers associated with genetic mutation or deletions are also targets of DNA-hypermethylated

silencing in sporadic cases of the same cancer type [23].

Two recent studies, utilizing genome-wide DNA analyses in cancer have suggested that only a minority of DNA-methylation changes map to promoter regions. One study reported that DNA-methylation changes in colorectal cancer do not occur directly at transcriptional start sites or CpG islands, but in the 2 kb flanking sequence of CpG islands, in regions termed CpG island shores [24]. A second study also found that the majority of DNA-methylation changes do not occur at gene promoters but in introns or intragenic regions [25]. It is possible that these changes may disrupt normal non-coding RNA expression, such as microRNAs (miRNAs). Several miRNAs have been shown to be epigenetically silenced, and may contribute to cancer development and metastasis [26].

EPIGENETICS AND GENOMIC IMPRINTING

Genomic imprinting is the parent-of-origin specific monoallelic transcriptional silencing observed in placental mammals [27]. These specific transcripts constitute a particularly interesting example of epigenetic regulation, since in an individual cell there are active and repressed alleles of the same gene. The allelic differences in transcriptional activity originate from the distinct patterns of differential DNA methylation at CpG dinucleotides, established in the male and female gametes and maintained throughout somatic development [28]. This oocyte-specific DNA methylation is carried out by a DNMT3L/DNMT3A complex, which requires the total removal of H3K4 methylation at the site of DNA methylation by AOF1/KDM1B [29].

Regions that are differentially DNA-methylated regions (DMRs) in the germline are referred to as the primary mark, or imprinting control region (ICR). Some DMRs are established only in developing tissues, and are called secondary or somatic marks [27]. The presence of single ICRs within imprinted domains suggests co-ordinate regulation of several genes by a single *cis*-acting control element [30]. These regions of differential DNA methylation are often, but not exclusively, associated with differential chromatin modifications. Methylated alleles are coupled with repressive chromatin modifications such as H3K9me2/3, H4K20me3 [31–33], as well as H4R3me2D [34]. The unmethylated alleles of DMRs are coupled with permissive chromatin modifications including H3K9ac and H3K4me2/3 [35] (Figure 1). Recently it has been shown that a number of imprinted genes, not associated with differential DNA methylation in their own promoter, have allelic histone modifications which are required for maintaining somatic imprinting [31, 32, 36, 37]. To date, all the biochemical components that have been associated with genomic imprinting have further roles in expression and silencing of non-imprinted genes during differentiation [2], i.e. their regulation does not seem to require unique activators or repressors.

IMPRINTING AND CANCER

Several imprinted genes undergo loss of imprinting (LOI) in cancer, such that both alleles may be transcriptionally active and so biallelically expressed, or the gene becomes completely silenced. This loss of imprinted gene expression is considered the most abundant and precocious alteration in cancer [38] (Table 1). The case for an involvement of imprinted genes in the aetiology of cancers is supported by the fact that many cancer-associated cytogenetic abnormalities show parent-of-origin effects. For example, somatic chromosomal events leading to maternal loss-of-heterozygosity (LOH) of chromosome 11p15 occurs in 30–50% of Wilms' tumours [39–41] and is frequent in other cancers [42]. Additional evidence endorsing the involvement of imprinted genes in cancer comes from the reports of biallelic expression of *IGF2*, and the reciprocal silencing of *H19* due to hypermethylation of the *H19*-differentially methylated domain (DMD), in a subgroup of Beckwith–Wiedemann syndrome patients who have a greatly elevated risk of developing Wilms' tumour [43].

THE FUNCTION OF IMPRINTED GENES IN CANCER TRANSFORMATION

Several imprinted genes have been shown to be key embryonic regulators, with paternally expressed genes enhancing growth, whereas maternally expressed genes limit it [44]. The majority of imprinted genes are highly expressed during *in utero* development, after which expression declines. However, the expression of some imprinted genes has been shown to have either oncogenic or tumour suppressing

Table I: A list of the human imprinted domains that commonly show LOI and DNA-methylation changes in cancer

Imprinted region	Chromosomal location	Cancer type
DIRAS3	1p31.1	Hepatocellular and follicular thyroid carcinomas; oligodendroglial, ovarian and breast tumours; multiple myeloma
ZAC1	6q24.2	Gastric adenocarcinoma; renal cell carcinoma; ovarian and breast tumours
GRB10	7p12	Cervical squamous cell carcinoma
PEG10	7q21	Hepatocellular carcinoma, B-cell leukeamias
MEST	7q32.2	Adrenal carcinoma; osteosarcoma; lung and breast tumours
KvDMR1 (CDKN1C)	11p15.5	B-cell lymphomas and leukeamias; gastric cell, breast, colorectal, pancreatic, prostate and bladder cancers; hepatocarcinoma; Wilms and rhabdoid tumours; laryngeal squamous carcinoma
H19-IGF2	11p15.5	Colorectal, prostate, pituitary, lung, cardiac, pancreatic, yolk sac, bladder, ovarian and testicular germ cell cancers; Wilms tumour, hepatoblastoma; head/neck, cervical and nasopharyngeal carcinoma; adrenocortical tumour; leukaemias and lymphomas
DLK1-DIO3	14q32.2	B-cell malignancies; multiple myeloma; pituitary adenoma; hepatocellular and renal cell carcinoma; Wilms tumour; neuroblastoma; gilomas
SNURF/SNRPN	15q11.2	Ovarian and gonadal/germ cell tumours
PEG3	19q13.43	Gliomas; ovarian tumour; oligodendroglimas
NNAT	20q11.23	Pituitary adenoma; neuroblastoma; acute leukeamias
GNAS	20q13.32	Leukaemias; pituitary, esophageal and colorectal cancers.

activity if expression persists into adulthood. Probably the key oncogenic imprinted gene is the paternally expressed *IGF2*, a potent growth enhancer. Biallelic expression of this gene has been reported in the majority of cancer types investigated (Table 1). The imprinting of *IGF2* is dependent on a complicated regulatory mechanism that utilizes multiple enhancers, boundary elements, histone modifications and complex physical DNA looping, all of which are allele-specific [45–49]. The 3D conformation and allelic-specific expression require the correct allelic DNA methylation at the *H19*-DMD. This DMR is DNA-methylated on the paternal allele, and the unmethylated maternal allele is associated with CTCF/cohesin insulator binding that coordinates the long-range intrachromosomal interactions [50, 51], which have recently been shown to be lost in human cancer cells [52]. The *H19* non-coding RNA has recently been shown to have tumour-suppressing activity, and be a precursor RNA for miR-675 [53]. This interesting observation suggests that not only are the cancer associated effects of *H19*-DMD DNA-hypermethylation due to biallelic *IGF2* expression, but maybe also be attributed to other genes up-regulated in the absence of miR-675.

The maternally expressed tumour suppressor gene *CDKN1C* also maps to human chromosome 11 [54], within a second imprinted sub-domain [55, 56], ~700 kb centromeric to *IGF2/H19*. The paternal allele silencing of *CDKN1C* is under the control of the long non-coding *LIT1/KCNQ1OT1* RNA that originates from the differentially DNA-methylated KvDMR1. Hypomethylation of the KvDMR1 and the subsequent silencing of *CDKN1C*, a mechanism that causes in Beckwith–Wiedemann syndrome, frequently occurs in sporadic cancers [57–59]. Loss of maternal expression of *CDKN1C* in cancer has also been shown to be due to loss-of-heterozygosity and promoter hypermethylation of the *CDKN1C* gene itself [60, 61]. The *CDKN1C* gene is a member of a family of enzymes that negatively regulate the action of cyclin-dependent kinases, which ultimately controls cell-cycle progression [54, 61]. The cyclin-dependent kinase inhibitors (CDKNs) family of genes are strong tumour suppressors that include *CDKN1A* (*p21CIP*), *CDKN1B* (*p27KIP1*) and *CDKN1C* (*p57KIP2*), with the latter being a negative regulator of G1 cyclin/CDK checkpoint complexes [62, 63].

Additional imprinted genes are known to be involved in the regulation of the cell cycle. The imprinted *BLCAP* gene, mapping to human chromosome 20 [64] is a tumour suppressor that limits cell proliferation and stimulates apoptosis [65] and *ARH1* (*DIRAS3*) on chromosome 1 is involved in G1 phase progression [66]. Both of these genes are downregulated in numerous cancers [67, 68]. Recently, the tumour-suppressor gene *RB1* has been shown to be imprinted, with the full-length transcripts showing an expression bias from the maternal allele [69]. The phosphorylation status of RB1 is an important

factor in controlling G1-S phase transition of the cell cycle. Interestingly, inhibitors of cyclin-dependent kinases, such as *CDKN1C*, play an important role in this process, suggesting that both of these maternally expressed genes are potent inhibitors of cell proliferation and act within the same pathway.

IMPRINTED DMR METHYLATION CHANGES IN CANCER

It has been well documented that the DNA-methylation profile of individual ICRs, such as *H19*-DMD, is altered in numerous cancer types (Table 1), but it remains to be determined whether collectively, all DMRs are equally susceptible to aberrant DNA methylation observed in cancer, or whether certain regions of differential methylation are more prone to cancer associated DNA-methylation changes, such as those DMRs that acquire their DNA methylation somatically. Of the approximately 60 imprinted genes in human, around half have been shown to be subject to LOI in cancer. However, the precise mechanism of LOI in these cases has not been investigated. No studies to date have tried to systematically correlate the loss of epigenetic information (both DNA methylation and histone modifications) at all DMRs with genome-wide DNA-methylation status of non-imprinted genes within specific cancer types. These complex analyses would identify if there is a common mechanism involved in the epimutations of DMRs, and whether they are associated with certain driver mutations (for example V600E *BRAF* activating mutation or loss of *CDKN1B/p27KIP1* and *MLH1*), microsatellite instability (MSI) or CpG island methylator (CIMP) phenotypes [70–73]. These genome-wide studies are lacking partly due to the unsuitable molecular methods for determining the DNA-methylation profiles of imprinted DMRs, and to the complexity of reliable allelic quantification.

In addition, it would also be interesting to determine whether the DNA-methylation profile of tumour suppressor genes, working within the same physiological pathways, are prone to epimutations, or whether the hypermethylation of genes within a pathway are mutually exclusive, as is the case with coding mutations within the *EGFR/KRAS* and *CDKN2A/RB1* genes [74, 75]. Utilizing genome-wide methylation approaches in cancer samples would also allow investigators to determine whether epimutations are mutually exclusive, endorsing the theory that once a pathway is disrupted by a mutation in one gene, additional hits do not provide selective advantage for the tumour cells.

MOLECULAR APPROACHES TO DETERMINE DNA METHYLATION

The full extent of the effects of global DNA-methylation changes on transcription and chromatin organization remains unknown. Technical challenges have been partially overcome with the recent availability of high-density arrays, and massive parallel sequencing technologies. However, the majority of these new methods do not allow for the simultaneous identification of hypermethylated genes and allele-specific quantification essential for assessing changes in imprinted DMR DNA methylation. In most cases reliable allelic quantification is at the expense of the number of regions that can be analysed. Methods that have previously been employed to determine DNA methylation at DMRs include Southern blotting, methylation specific PCR (MS-PCR), COmbined Bisulphite and Restriction Analysis (COBRA), methylation-sensitive single nucleotide primer extension (ms-SNuPE), pyrosequencing, PCR melt-curve analysis and traditional cloning and direct sequencing of individual DNA molecules (Table 2). All these methods, with the exception of Southern blotting, rely on bisulphite conversion of DNA, where unmethylated cytosines are efficiently deaminated to uracil in single stranded DNA, and methylated cytosines remain unchanged, during subsequent PCR amplifications. The drawback to all of these commonly used techniques is that they are costly and time consuming and PCR of bisulphite-treated DNA is difficult, and can introduce preferential amplification artefacts.

In recent years chromatin immunoprecipitation (ChIP) approaches have allowed researchers to investigate interaction between proteins and DNA, and this has been extended to assess the genomic localization of both methylated DNA and the plethora of histone modifications for which antibodies are available. Methylated DNA immunoprecitation (meDIP) is a ChIP-based method that uses either an antibody directed against 5-methylcytosine or MBD protein (methylation binding domain protein) such as MBD2B [76] to enrich methylated DNA fragments [77]. The meDIP-enriched fragments can then be subjected to PCR, hybridization to tiling arrays or massively parallel sequencing. This technique allows

Table 2: A list of the commonly used protocols utilized for DNA methylation analysis, each with a brief overview of the technique and the benefits and drawbacks for assessing imprinted DMR methylation status

Technique	Protocol	Pros and Cons
Southern blot analysis	Digestion of DNA with methylation-sensitive restriction enzymes and subsequent resolution of fragments on agarose gel. After DNA transfer to a filter, the digested DNA is hybridized with a labelled probe.	X Time consuming. X Requires large amounts of DNA. X Generally probes are radioactively labelled. √ No chemical conversion required. √ Quantitative.
Me-DIP (methylated-DNA immunoprecipitation)	Fragmented methylated DNA is precipitated with antibodies specific to 5-methyl cytosine or methyl-binding proteins. Enriched DNA is subject to PCR, hybridization to genome tiling arrays or next generation sequencing.	X Over representation of repetitive sequences masking unique genomic sequences with lower methylation content. √ No chemical conversion required. √ Can use as little as 10 ng of DNA
MS-PCR (methylation-sensitive PCR)	PCR primers designed that differ only in TpG or CpG content, allowing subsequent methylation specific amplification.	X Primers can misprime leading to inaccurate results. X Qualitative at best. √ Easy to perform, requiring no special equipment.
COBRA(COmbined Bisulphite Restriction Analysis)	The restriction digestion of bisulphite PCR products with enzymes that recognize CpGs. After PCR and digestion, unmethylated sites (TpG) are not digested.	X limited to assess single sites within PCR products. X Can use only a limited number of restriction enzymes, such as TaqI (TCGA), TaiI (ACGT) and BstUI (CGCG). X Incomplete digestion manifests as unmethylated DNA. X Qualitative at best. √ Easy to perform, requiring no special equipment.
Ms-SNuPE (methylation-sensitive Single NUcleotide Primer Extension)	Bisulphite PCR products are denatured and an internal oligonucleotide used to prime an extension reaction to determine the presence of a thymidine or cytosine nucleotide at specific CpG site.	X Restricted to single nucleotide resolution. X Requires radiolaelled dCTP and dTTP. √ Quantitative.
Bisulphite pyrosequencing	After bisulphite PCR, the base composition is determined by sequential addition of nucleotides in the presence of an enzyme/substrate mix that converts every integrated nucleotide into a light signal.	X Limited to small PCR product. √ Quantitative, with signal intensity correlating to nucleotide incorporation. √ Can be used for high-throughput studies.
Direct sequencing of PCR products	After bisulphite PCR, the products are cloned into vectors and transformed into bacteria. Individual clones represent individual DNA molecules	X Large number of clones required to determine methylation profile. X Large analytical effort. √ Can determine allele-specific methylation patterns.
ChIP-seq-2nd generation sequencing. Solexa (Illumina), 454 (Roche), ABI SoLiD (applied Biosystems).	The unbiased sequencing of millions of small fragments generated from a bisulphite converted DNA template	X Expensive. X Only short reads generated (60–300 bp). X Requires complex algorithms to assemble sequence, which has additional complexity due to only three bases present in unmethylated DNA, making aligning difficult. √ (Semi-) quantitative.
Commerical methylation-arrays	Bead-arrays that allow the DNA-methylation profile of 0.1% of CpG to analysed in a single sample	X Expensive. X Only single CpG dinucleotides analysed per region. X Many sites that show changes in cancer are not included on array (i.e. CpG island shores). X Currently only available for humans. √ Quantitative. √ Can analyse up to 14 000 genes in a single reaction. √ Results comparable to other techniques.

for interrogation of the complete genome, but has limitations due to the majority of detectable DNA-methylation changes being associated with repeats and non-CpG island regions [78].

HIGH THROUGHPUT ANALYSIS OF ALLELIC-SPECIFIC EXPRESSION

There are now many array- based approaches that allow for analysis of the allele-specific expression of genes, many of which are based on high-throughput genotyping arrays. For example, the HuSNP oligonucleotide arrays from Affymetrix have successfully been used to quantitate allelic expression at both imprinted and non-imprinted genes, highlighting that allelic variation can contribute to the variation in heritable traits [79]. There are now several reports in literature describing the use of custom genotype approaches for determining allele-specific gene expression of highly polymorphic single nucleotide polymorphisms (SNPs) within selected genes. Using such approaches several novel imprinted genes have been identified [80, 81]. Daelemans *et al.*, recently used two different quantitative genotype technologies to screen for novel imprinted genes in human placenta, choosing SNPs that mapped within genes with previously reported preferential expression or genes predicted to be imprinted from bioinformatics studies [82]. In this study, the authors conclusively show that both the Sequenom Mass Spectrometer and Illumina Beadarray ASE platforms are sensitive enough to detect strong allelic skewing and were able to confirm the imprinting status of 18 known imprinted genes.

QUANTITATIVE DNA-METHYLATION TECHNOLOGIES

These new advances in genotyping technology have recently been applied to detecting DNA-methylation changes at base-pair resolution using high multiplex mediated PCR of bisulphite converted DNA. Two high-throughput, quantitative, commerical DNA-methylation platforms widely used by researchers are the Illumina GoldenGate and the Infinium arrays (Illumina Inc., San Diego, CA). The GoldenGate methylation Cancer Panel 1 array is based on methylation-specific, ligation mediated amplification, and simultaneously analyses 1505 CpG dinucleotides from 807 genes, including 67 CpG sites associated with 29 imprinted genes, with 11 CpGs mapping to DMRs. The Infinium methylation assay, based on methylation-sensitive single base pair extension, analyses 27 578 CpG sites covering more that 14 000 genes, including 30 imprinted genes and 15 DMRs. Despite the relatively large number of CpG dinucleotides analysed on these commercial arrays, the specific regions assessed do not usually map to regulatory features of DMRs, such as CTCF-binding sites, with the majority mapping to alternative promoters located in unmethylated CpG islands. In addition, these commercial DNA-methylation arrays do not assess DMRs that have low CpG content that do not fulfil the classical CpG island criteria, such as the IG-DMR on human chromosome 14 and the *IGF2*-DMR0 on chromosome 11 [83, 84]. Despite these drawbacks, these arrays are invaluable for cancer research, and can generate huge amounts of data allowing for the comparison of different DNA-methylation profiles from many different tissue types. Like the genotype arrays, these technologies offer researchers the chance to custom-optimize the platform to target specific genes.

In conclusion, despite much effort to identify imprinted genes in humans, the precise role of these transcripts in cancer, and the mechanism leading to LOI are unknown. It is therefore only a matter of time before custom DNA-methylation arrays are available that specifically target all imprinted loci and allow investigators to fully determine the Imprintome of single DNA samples.

Key Points

- Genomic imprinting is an epigenetic mechanism of transcriptional control that results in parent-of-origin monoallelic expression.
- LOI, via aberrant DNA methylation, frequently occurs in all types of cancer.
- In the future it will be necessary to improve genome-wide techniques to quantitate allelic DNA methylation to allow the mechanisms of LOI to be deciphered in cancer.

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