The history of cancer epigenetics

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Since its discovery in 1983, the epigenetics of human cancer has been in the shadows of human cancer genetics. But this area has become increasingly visible with a growing understanding of specific epigenetic mechanisms and their role in cancer, including hypomethylation, hypermethylation, loss of imprinting and chromatin modification. This timeline traces the field from its conception to the present day. It also addresses the genetic basis of epigenetic changes — an emerging area that promises to unite cancer genetics and epigenetics, and might serve as a model for understanding the epigenetic basis of human disease more generally.

Epigenetic inheritance is defined as cellular information, other than the DNA sequence itself, that is heritable during cell division. There are three main, inter-related types of epigenetic inheritance: DNA methylation, genomic imprinting and histone modification. This timeline traces the field from its conception to the present day. It also addresses the genetic basis of epigenetic changes — an emerging area that promises to unite cancer genetics and epigenetics, and might serve as a model for understanding the epigenetic basis of human disease more generally.

Hypomethylation and gene activation

Loss of DNA methylation at CpG dinucleotides was the first epigenetic abnormality to be identified in cancer cells. At a symposium at Johns Hopkins in 1982 on tumour-cell heterogeneity, Andy Feinberg and Bert Vogelstein wondered what mechanism accounted for high-frequency 'mutations', adaptation to tumour microenvironment and plasticity in some cancers. The conference was organized by Donald Coffey, who had introduced the two investigators. At the time, many groups were excited by observations that DNA methylation might be linked to tissue-specific gene silencing, so Feinberg and Vogelstein searched for differences between cancers and normal tissues. They used Southern blotting to analyse DNA that had been digested with methylation-sensitive restriction enzymes and found that a substantial proportion of CpGs that were methylated in normal tissues were unmethylated, then one will only observe methylation only at sites that are normally methylated in experimental design; if one looks for altered methylation only at sites that are normally unmethylated, then one will only observe hypermethylation. The frequency of hypomethylated sites might be quite high, as indicated by high-throughput genomic-methylation analysis of tumours, including cancers of the stomach, kidney, colon, pancreas, liver, uterus, lung and cervix. Strong support for hypomethylated gene activation leading to activation of genes that are important in cancer includes promoter CpG demethylation in the overexpression of cyclin D1 and maspin in gastric carcinoma, MN/CA9 overexpression in human renal-cell carcinoma, S100A4 metastasis-associated gene in colon cancer and human papillomavirus 16 (HPV16) expression in cervical cancer. Extensive observatio...
Box 1 | The three main types of epigenetic information

Cytosine DNA methylation is a covalent modification of DNA, in which a methyl group is transferred from 5-adenosylmethionine to the C-5 position of cytosine by a family of cytosine (DNA-5)-methyltransferases. DNA methylation occurs almost exclusively at CpG nucleotides and has an important contributing role in the regulation of gene expression and the silencing of repeat elements in the genome.

Genomic imprinting is parent-of-origin-specific allele silencing, or relative silencing of one parental allele compared with the other parental allele. It is maintained, in part, by differentially methylated regions within or near imprinted genes, and it is normally reprogrammed in the germline.

Histone modifications — including acetylation, methylation and phosphorylation — are important in transcriptional regulation and many are stably maintained during cell division, although the mechanism for this epigenetic inheritance is not yet well understood. Proteins that mediate these modifications are often associated within the same complexes as those that regulate DNA methylation.

Recent studies of pancreatic cancer by Goggins and colleagues showed widespread hypermethylation associated with proliferation-linked genes, including 14-3-3σ10,17. Second, a cellular 'methylator phenotype' has been linked to mismatch repair, first by Lengauer and colleagues, who showed that cancer cells that are deficient in DNA mismatch repair silenced retroviral construct promoters by DNA methylation19. This observation was challenged by Jones and colleagues20 and the methylator phenotype concept (about which more below) will probably be debated until its genetic basis is elucidated. Nevertheless, the idea makes sense, as hypermethylation of the mismatch-repair gene MLH1 is commonly found in mismatch-repair-defective tumours, as first described by Kolodner’s group21. In addition, abnormal imprinting (discussed below) is also more commonly found in mismatch-repair-defective colorectal cancers22.

Timeline | Milestones in human cancer epigenetics

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Third, Ehrlich and colleagues recently linked tumour hypomethylation in cancer to chromosomal instability. Hypermethylation is particularly severe in pericentromeric satellite sequences, and several cancers (Wilms tumour, ovarian and breast carcinoma) frequently contain unbalanced chromosomal translocations with breakpoints in the pericentromeric DNA of chromosomes 1 and 16 (REF. 23). These rearrangements are specific, and not due to global genomic instability — in Wilms tumours, t(1;16) translocations are sometimes the only detectable abnormality. These unbalanced translocations produce loss of heterozygosity (LOH) for markers on chromosome 16, which, in turn, strongly correlates with tumour anaplasia24. Demethylation of satellite sequences might predispose to their breakage and recombination. The presumed causal relationship in these cancers has not been proven, but a developmental disorder — ICF syndrome (immunodeficiency, chromosomal instability and facial anomalies) — was found by several investigators to be caused by loss-of-function mutations in the cytosine DNA methyltransferase Dnmt3b25–27. ICF syndrome has as its cardinal features loss of methylation in classical satellite DNA and mitogen-inducible formation of bizarre multiradial chromosomes that contain arms from chromosomes 1 and 16 (REF. 26). Indeed, this is why Dnmt3b was successfully scrutinized as a candidate gene for the disorder by Vieges-Pequignot and Bestor, Gartler, Li and others. Nevertheless, ICF syndrome does not lead to cancer (discussed below). An even more direct link between hypomethylation and chromosomal instability was made by Jaenisch’s group, who found that neurofibromatosis 1 (Nf1)+/–Trp53−/− mice showed a 2.2-fold increase in frequency of LOH when a hypomorphic Dnmt1 allele was introduced28. Another potential connection between hypomethylation and chromosomal instability is the hypomethylation of L1 retrotransposons in colorectal cancer, which might promote chromosomal rearrangement29.

Fourth, hypomethylation is a mechanism of drug-, toxin- and viral effects in cancer. In addition to gene amplification, hypomethylation of the multidrug-resistance gene Mdr1 correlates with increased expression and drug resistance in acute myelogenous leukaemia30. Toxic carcinogens might also act through methylation alterations. For example, cadmium induces DNA methyltransferase activity and leads to acute hypomethylation, which is followed by hypermethylation of DNA after chronic exposure to this epigenetic carcinogen31. Similarly, arsenic induces Ras hypomethylation in mice32. Finally,
cervical cancer latency seems to be caused, in part, by hypermethylation of the HPV16 genome, and latent Epstein–Barr virus in human lymphoma cells uses a similar strategy to enforce silencing of a subset of its genes. In cervical cancer, activation of the HPV genome and progression occur with progressive hypermethylation of the virus in precursor lesions.

An exciting recent development in cancer biology is the link between diet and cancer. A common polymorphism of methylenetetrahydrofolate reductase (MTHFR), which is involved in the biosynthesis of the methylation precursor S-adenosylmethionine, was associated with an increased colorectal cancer prevalence in a population-based study, and cancer incidence was lower in patients with a high methionine diet. Increased colorectal cancer prevalence was also linked to alcohol consumption and colonic hypomethylation. A hypermethylation complex with human cancer is unambiguous. As uncovered by Delattre and colleagues in 1998, germline and somatic mutations in SNF5 (also known as INI1) — which encodes a SWI/SNF complex component — cause rare, but lethal, cancer — malignant rhabdoid tumour (mRT). It is not yet known if, as might be predicted, this class of neoplasms has more extensive global demethylation than most cancers. The more general question, whether SWI/SNF function is altered in more common types of cancers, is also unanswered, but results from research on the retinoblastoma (RB) tumour suppressor (see below) indicate that this is indeed the case.

A second chromatin protein that has been linked to hypermethylation and cancer was recently identified by Muggia and colleagues, who found that Lsh, a SNF2-family member, is required for maintenance of normal methylation. Gene knockout leads to a global defect in genomic methylation, as well as a severe proliferative defect and chromosomal instability. Further support for a link between hypermethylation and tumorigenesis was provided by Hirshoashi's group, who identified a common splice variant of DNM3B in patients with liver cancer, which is associated with hypermethylation and causes hypomethylation of pericentromeric satellite sequences when transfected into cells.

Hypermethylation and gene silencing

Of course, hypermethylation is not the only way in which methylation can contribute to cancer. Steve Baylin and Barry Nelkin, in conversations at Johns Hopkins with Feinberg and Vogelstein, decided to examine calcitonin, which is a marker of small-cell lung cancer and was their main interest at the time. In 1986, they were surprised to find site-specific hypermethylation of calcitonin, with relative silencing of calcitonin expression. However, calcitonin is not a tumour-suppressor gene, and the first link between hypermethylation and tumour-suppressor genes was made, fittingly enough, on the first known tumour-suppressor gene — the retinoblastoma gene RB. This gene might not come to mind as a locus that is frequently inactivated by the epigenetic pathway, but, in fact, the RB promoter is methylated in a significant subset of sporadic and even hereditary retinoblastomas. The papers reporting this phenomenon, published by the Dryja and Horstemke laboratories, were the first to indicate that tumour-suppressor silencing might occur by an epigenetic pathway. In 1991, Dryja's group showed that the hypermethylation was confined to one allele, again indicating specificity. He argued explicitly that it leads to gene silencing. Direct confirmation of epigenetic silencing of a tumour-suppressor gene was provided by Sakai's group in 1993, who showed a 92% reduction of RB expression in tumours with promoter hypermethylation and by Horstemke's group in 1994 (REF 46).

Two years later, beginning in 1995, several groups, including the Baylin, Jones and Sidransky laboratories, confirmed promoter hypermethylation at numerous other loci in cancer cells, supporting the principle of epigenetic gene inactivation in cancer. Key tumour-suppressor proteins — including the INK4A (also known as p16; encoded by CDKN2A) cyclin-dependent kinase inhibitor, the mismatch-repair enzyme MLH1, the von Hippel–Lindau (VHL) tumour suppressor and E-cadherin — were all shown to be eliminated both in cell lines and in primary cancers by an epigenetic pathway that correlates with dense CpG methylation of their gene promoters. The primary publications that describe these correlations for the CDKN2A and VHL genes appeared between 1994 and 1995 (REFS 47–50) and were extended to include MLH1 between 1997 and 1998 (REFS 21,51,52). It is important to note that the basic relationship between CpG island methylation and gene inactivation, and the identification of CpG islands themselves, came from early studies of X chromosome inactivation (BOX 2).

After leaving the Baylin laboratory, J.-P. Issa (and colleagues) then showed methylation profiling data that indicated a dichotomous classification of human carcinomas into frequent promoter methylation and infrequent methylation groups, and led him to promote the idea of a CpG island methylator phenotype — termed "CIMP P" — in human cancer. This attractive, but still controversial, concept has stimulated many follow-up studies and, as we discuss below, there are now several candidate biochemical mechanisms that could conceivably account for CIMP P. The first functional report showing a relationship between tumour-suppressor activity and DNA methylation was performed by West and Barrett in 1993, in which they examined a model of progressive loss of tumour-suppressor activity in Syrian hamster cells.

[Diagram showing the relationship between methylation and cancer]

Epigenotype-phenotype analysis shows gatekeeper role of LOI in a human cancer syndrome

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In the background of the discoveries that link CpG-island methylation and gene inactivation is a large body of important correlative and mechanistic data that are related to X chromosome inactivation. For example, an early clue to a role in gene silencing came from studies of Mohandas et al., who showed in 1981 that 5-aza-2'-deoxycytidine (5-azaCdR), an inhibitor of DNA methylation, could reactivate the inactive X chromosome. The discovery of the functional significance of what are now termed CpG islands also came from studies of the X chromosome. Wolf, Migeon and colleagues showed in 1984 that clusters of CpG dinucleotides are specifically methylated on the inactive X chromosome and reactivated with 5-azaCdR. These were later extensively characterized and termed CpG islands by Adrian Bird and colleagues, who found that they were common in the promoters of autosomal genes. Studies from the Gartler laboratory showed that gene reactivation on the inactive X chromosome is associated with large regions of promoter demethylation after 5-azaCdR treatment, indicating a causal relationship between methylation and gene silencing on the inactive X chromosome.

Incidentally, a peculiarity of the DNA methylation literature is the term CpG: what else connects two sugars but a phosphate? After all, we don’t say ‘TpApTpA box’!

The connection between epigenetic gene silencing and chromatin modifications, another theme that is increasingly important in the progress of cancer epigenetics, was also highlighted early on in studies of X inactivation — with memorable images of 45 human chromosomes intensely stained by an antibody specific for acetylated histone H4, with the lone inactive X chromosome globally deacetylated and unstained.

That DNA methylation is causal in maintaining the silent epigenetic state has been shown by the potency of demethylating drugs in reactivating gene expression and by recent studies that use somatic-cell knockout procedures, or antisense and RNA interference, to eliminate DNA methyltransferases from cancer cells. These manipulations resulted in tumour-suppressor gene reactivation, but the details differed depending on the experimental system: acute elimination of DNMT1 in HCT116 cells by antisense or RNA interference was sufficient to reactivate CDKN2A, whereas in this same cell line, a double somatic knockout of both DNMT1 and DNMT3B was required to demethylate and reactivate this gene. A possible explanation is that DNMT3B can ultimately replace the function of DNMT1 during cell selection, as occurs in knockout experiments, but DNMT3B does not completely substitute for DNMT1 acutely.

What has not been as clear is that the mechanism of initial silencing of these genes is hypermethylation, and this has been the subject of some debate: see, for example, Bestor. Indeed, Modrich and colleagues showed that activation of M.H1 by 5-azaCdR is rapidly reversed spontaneously. So, methylation changes could arise secondarily to other epigenetic changes, such as chromatin modification, but then help to maintain the silenced state. Consistent with this idea, although tumour-suppressor gene silencing per se can be a dominant trait in somatic-cell genetic experiments, a trans-acting defect in methylation has not been demonstrated in tumour cells and the same is as true for hypomethylation as for hypermethylation. Indeed, a significant challenge to the causal role of hypermethylation in initiating the process of gene silencing comes from a recent report showing that methylation of histone H3 lysine 9 — that is, chromatin modification — occurred in conjunction with re-silencing of CDKN2A in the absence of DNA methylation, in cells in which CDKN2A had previously been activated by DNA methyltransferase knockout. Consistent with this observation, Clark and Melki also point out cogently that CDKN2A is silenced in proliferating colonies of mammary epithelial cells that escape senescence, even in the absence of promoter methylation, suggesting that hypermethylation is not responsible for silencing, but helps to maintain silencing.

Clearly, one must look at methylation in cancer as an example of epigenetic dysregulation, with both hypomethylation and hypermethylation having significant roles. Nicely summarizing this situation, a recent study of Wilms tumours found different unique-gene loci that were affected by hypomethylation or hypermethylation in the same tumour. Moreover, the final epigenetic programme varies strongly by tumour type; an interesting example is a ‘counterintuitive’ report of CDKN2A hypomethylation in some breast cancers compared with normal breast tissue.

Loss of imprinting in cancer

Imprinting — which refers to conditioning of the maternal and paternal genomes during gametogenesis, such that a specific parental allele is more abundantly (or exclusively) expressed in the offspring — was discovered in embryological studies published in the mid-1980s. But even earlier, cancer cytogeneticists studying two human neoplasms, hydatidiform moles and ovarian teratomas, had produced data that presaged these findings. M oles — which are placenta-derived (that is, extraembryonic) tumours — were found to contain two complete sets of paternal chromosomes with no maternal contribution, whereas findings in both mice and humans indicated that ovarian teratomas, which contain many tissue types, but never placental trophoblast, carried a bi-maternal chromosome complement.

Evidence for a role for human imprinted genes in development then came from two neurodevelopmental disorders, Prader–Willi syndrome (PWS) and Angelman syndrome (AS). These were found to be caused by uniparental chromosomal disomy of the long arm of chromosome 15, which were maternal in PWS and paternal in AS. Moreover, to this timeline, in the late 1980s, several independent studies reported a strong (in fact, absolute) parent-of-origin bias in LOH for chromosome 11p15 alleles in Wilms tumours and embryonal rhabdomyosarcomas, with invariable loss of maternal and duplication of paternal alleles. This striking finding was hard to explain without posulating a role for imprinted gene(s) in these tumours, as was suggested first by Sapienza and colleagues in 1989. An important clue was also provided by studies of the disorder Beckwith–Wiedemann syndrome (BWS), which causes prenatal overgrowth, birth defects (including a large tongue, ear creases and abdominal-wall closure defects) and predisposition to various embryonal tumours of childhood including Wilms tumour. Rare familial cases of BWS also indicated a parent-of-origin effect, as the overgrowth phenotype was only seen after maternal transmission.

More direct evidence came from mapping of BWS to 11p15, followed by observations from M annens et al. that chromosomal rearrangements in 11p15 in patients with BWS were all of maternal origin. The discovery of bona fide human imprinted genes was made independently in the early 1990s by Tycko, Ohlsson, Feinberg Polychronakos and Reeve (and colleagues), following from mouse studies of these same genes — IGF2 and H19. Additional imprinted human genes were uncovered in the PWS/AS region of chromosome 15; studies of the mechanism of imprinting were in full swing by the mid-1990s, with some landmarks being the direct demonstration of a
embryonal tumours is invariably linked to a gain of DNA methylation that is localized to the 5′ sequences and transcribed region of the closely linked and reciprocally imprinted H19 gene, which is thereby transcriptionally silenced90,91. The presence of H19 hypermethylation (a somatic gain of an epigenetic mark on the previously expressed maternal allele) was found not only in the tumour DNA, but also in the non-neoplastic kidney parenchyma surrounding some of the tumours90. These observations were the first to indicate a gatekeeper role for epigenetic alterations in cancer, as they are the earliest observable genetic change (FIG. 1). In 1997, mosaicism for H19 hypermethylation in patients with Wilms tumour was confirmed in an independent series of cases92. The net effect of this epigenetic lesion is silencing of H19, a gene that encodes an abundant spliced but non-translated RNA, and a reciprocal increase in expression of IGF2 (REFS 90,91). The roughly twofold increase in effective IGF2 gene dosage is now considered the most likely explanation for the associated tumour susceptibility, although H19 RNA is growth suppressive in some cancer cell lines93. The mechanism by which IGF2 promotes tumour formation might be by inhibiting apoptosis, as Hanahan and colleagues found that knockout of one allele (preventing biallelic progression) arrests tumour progression in an activated T-antigen transgenic tumour model94, and this arrested progression involves increased apoptosis95.

Epidemiological support for the gatekeeper role of altered methylation and LOI in Wilms tumour came from the discovery, in 2000, that Knudson’s hypothesis did not explain the bimodal age distribution of most Wilms tumours. Tumours that are “late arising”, that is, not in infancy, were found to involve epigenetic rather than genetic alterations, and early-arising tumours had classical genetic changes involving Wilms tumour 1 (WT1) and LOH96. Definitive clinical evidence for a gatekeeper function of the gain of DNA methylation upstream of H19 and LOI of IGF2 in Wilms tumours has since come from studies of tumour susceptibility in the pre-neoplastic disorder BWS (see below).

Indicating generality of a role for imprinted genes in cancer, a recent publication reported selective loss of paternal alleles (6/6 cases of LOH) on chromosome 19q in oligodendrogliomas97. As indicated by data from the Oshimura laboratory98, the putative tumour-suppressor gene identified by these data might be PEG3 — a paternally expressed imprinted gene that maps to band 19q13.4, or perhaps another nearby imprinted gene, as PEG3 might lie outside the region of LOH98. These results are provocative, as they seem to go against the “paternal allele active—growth promoter” paradigm. In fact, data from Peg3-knockout mice99 indicate the expected growth-promoting function of this paternally expressed gene (the knockout mice are small); but human PEG3 has tumour-suppressor activity in transfected cells100 and the gene is epigenetically silenced in gliomas101. Selective loss of alleles has also been reported in neuroblastomas102, but in these tumours the parent-of-origin dependence is by no means absolute and an unequivocally imprinted locus on chromosome 1 has not materialized. Recently, Morison, Reeve and colleagues have found selective loss of maternal alleles on chromosome 9p in childhood acute lymphoblastic leukaemias103. There are some data to indicate that LOI can also lead to tumour-suppressor gene silencing; for example, ARHI — a candidate breast tumour gene that was found by the Yu laboratory — shows aberrant allele-specific silencing104. In addition, LIT1 — an untranslated RNA found by the Feinberg, Oshimura and Higgins groups to undergo LOI in about half of patients with BW105,106 — might cause downregulation of CDKN1C (which encodes KIP2, also known as p57).

**Chromatin and methylation**

The third epigenetic mechanism — histone modification — has been the last to be linked to cancer research. A link between chromatin and DNA methylation, however, dates back to the 1980s, in the elegant observation by the Cedar and Graessmann laboratories that naked DNA templates, pre-methylated in vitro and then transfected or microinjected into cells, only became transcriptionally silenced after packaging into a repressive form of chromatin107,108. Proteins that bind to methylated CpGs were soon identified by Adrian Bird’s group109,110, and work from that laboratory, along with studies by Yaffe’s and Alan Wolfe’s groups, showed that these proteins (MECP2 and MBD2), as well as DNA methyltransferases themselves (DNMT1, DNMT3A and DNMT3B), physically associate with histone deacetylases111–115. DNMT1 maintains patterns of methylation during replication, and DNMT3A and DNMT3B can add methylation to previously unmethylated templates. A recurring theme of DNA methylation is that its machinery co-opts a more fundamental system of chromatin modification. For example, whereas DNMTs associate with chromatin proteins in mammals, the Dro sophila orthologues of methyl-binding proteins Mbd2 and Mbd3 do not bind methyl-C, but still show conserved
Moreover, Vogelstein and colleagues found transcriptional repressor function/histone deacetylase-binding cycle, which is present in organisms as diverse as yeast, Drosophila and humans, is an ancient mechanism for propagating epigenetic states, whereas the analogous CpG methylation/histone-deacetylase-binding cycle is evidently a later addition. This fail-safe mechanism, which stabilizes silent chromatin in mammals, keeps parasitic retroelements repressed and might have co-evolved with these invasive sequences.20

Another very recent advance that might prove relevant comes from observations by Henikoff and Ahmad that histones can be selectively replaced at transcriptionally active loci, in a manner that is independent of DNA replication12. This process entails the transcription-dependent accumulation of a highly conserved histone variant — H3.3 — which substitutes for the canonical H3 histone. Histone replacement, which presumably occurs after invasion of promoters by strongly activating transcription factors, offers an explanation for how the cell might reactivate genes that were previously silenced via histone methylation and then repressed within the 20q amplicon in breast cancer, and its overexpression is thought to impede normal CTCF binding22. It is intriguing that BORIS is itself a cancer/testis gene and, therefore, its hypomethylation might be linked to hypermethylation at other sites. A similar mechanism has been suggested by Chinnaiyan and colleagues for EZH2, an orthologue of the Drosophila chromatin-repressor protein ‘enhancer of zeste’. Increased expression of EZH2 is linked to generalized hypermethylation and gene silencing in metastatic prostate cancer23. As we discuss in more detail below, another methylation and chromatin connection involves RB, which associates both with DNA methyltransferases and with the SUV39H1 histone methyltransferase and H1P1, providing a mechanism for repressing cell-cycle genes, such as cyclin E.

BWS: epigenetic casualty in cancer

A key barrier to the acceptance of epigenetic alterations as a cause rather than a consequence of cancer, has been the lack, until recently, of well-defined human pre-neoplastic disorders that are caused by epigenetic mutations. There are known disorders involving genes that encode the methylation machinery of the cell (for example, DNMT3B in ICF syndrome and MECPT2 in Rett syndrome), but these disorders do not predispose to cancer. By contrast, many tumour-suppressor genes, when mutated in the germline, cause cancer predisposition syndromes (TP53 in Li–Fraumeni syndrome being the original).

However, the discovery of the mechanisms of BWS provides the genetic smoking gun for at least one epigenetic mechanism in cancer, genomic imprinting. The generalized overgrowth characteristic of BWS sometimes includes kidney enlargement, and the affected kidneys can contain persistent nephrogenic blastema — the precursor of Wilms tumour. By no means do all children with BWS develop Wilms tumours, but the relative risk is 816 (REF. 132). Potentially explaining this clinical heterogeneity, in the years between 1993 and 2000, BWS was shown to have various molecular causes, including LOI of IGF2 (REFS 90, 91, 133) or, alternatively, point mutations in the CDKN1C gene134 or epigenetic lesions in the nearby antisense RNA LIT1 (REFS 106, 107), which, together, lie within a separate imprinted subdomain of chromosome 11p15. Furthermore, a review of BWS cases in the literature up to 1999 indicated that cancer predisposition might be specifically associated with LOI of IGF2 and hypermethylation of H19 (REF. 135). To test this idea, several groups, including Mannens, Weksberg, Reik and Maher, found association of cancer in BWS and LOI of H19, although in those studies, because of patient numbers, one could not distinguish statistically an association specific to H19 from un parental disomy including H19 and other genes136–138. In the first epigenotype–phenotype study for any disease, DeBaun and Feinberg found
that in a large registry of patients with BWS that was studied prospectively, gain of methylation at H19, presumably resulting in biallelic expression of IGF2, was specifically and statistically associated with cancer risk, whereas loss of methylation at LIT1 was specifically associated with birth defects (macrosomia and midline abdominal-wall defects)\textsuperscript{139}. This specificity for cancer risk indicates a gatekeeper role of LOI in BWS (Fig. 1). The mechanisms accounting for gain of methylation at H19 or loss of methylation at LIT1 are not known, but recent findings by DeBaun, Feinberg, Maher and others\textsuperscript{140,141}, which associate BWS with in vitro fertilization, together with the simple fact that the epigenetic abnormality is widespread in various somatic tissues, indicates that these epigenetic aberrations occur very early in development, before the development of malignancy. In other words, epigenetic lesions in BWS are a cause, not a consequence, of cancer.

LOI might have a causal role in common cancer as well. A recent study indicates that, although BWS is rare, epigenetic alterations affecting IGF2 might be common in the general population and associated with more prevalent malignancies. LOI of IGF2 was found by Cui, Cruz-Correa and Feinberg in normal lymphocytes and colonic mucosa in 10% of healthy adults, and the odds ratio for LOI was 5.15 for patients with a positive family history, and 21.7 for patients with a past history, of colorectal cancer\textsuperscript{142}. Here too, the epigenetic abnormality is found in normal cells, and so is not an epiphenomenon of the cancer phenotype. Interestingly, the mechanism of LOI in colorectal cancer involves hypermethylation of IGF2, rather than hypomethylation of H19 that is seen in embryonal tumours\textsuperscript{128}, which is consistent with the idea that cancer is linked to epigenetic disequilibrium rather than hypomethylation or hypermethylation per se. The timing of epigenetic lesions in cancer is a topic that cannot be overemphasized. Promoter hypermethylation might also often be an early event. Ominously, in smokers with bronchial epithelial atypia that a pathologist would classify as pre-neoplastic, Herman and colleagues found already substantial hypermethylation of the CDKN2A promoter region\textsuperscript{143}. In an intriguing recent study, Sapienza has found familial clustering of epigenetic alterations involving H19, indicating that methylation might represent an epigenetic cancer-associated polymorphism in the population\textsuperscript{144}.

An alternative genetic argument can be derived from mouse models, with the caveat that mouse and human carcinogenesis might differ. Three mouse models potentially link DNA methylation to cancer. The first of these was the demonstration by Jaenisch and colleagues that a Dnmt1 hypomorphic mutation reduces the frequency of intestinal neoplasia when crossed to Apc\textsuperscript{min} in mice\textsuperscript{145}. These results indicate that hypomethylation might abate the risk of cancer, but more recent studies from Jaenisch and colleagues indicate just the opposite, with a high frequency of lymphomas in mice with a hypomorphic Dnmt1 allele\textsuperscript{146}. Together, the data indicate that a disruption in the balance of methylation is associated with cancer risk, an idea that is consistent with observations of human cancer. In a third model — heterozygous knockout of the Hic1 gene, which is hypermethylated in human colorectal cancer — Baylin and colleagues found a modest, but significant, increase in cancer frequency of very late onset, with hypermethylation of the wild-type allele\textsuperscript{147}.

**Tumour suppressors and chromatin**

The counter-argument from human genetic studies is that the many tumour-suppressor genes in cancer are not modifiers of DNA methylation, even though they are involved in virtually every other potential growth or regulatory pathway. On the other hand, many tumour-suppressor genes are involved in some aspect of chromatin structure. The first of these involved the cloning by Canaani, Rowley and Cleary of the ALL1 gene (also known as M LL or H R X) in 1991. ALL1 — which is altered by chromosomal translocations involving band 11q23 to produce fusion genes in human leukaemia\textsuperscript{148–150} — is the human homologue of Drosophila trithorax, which functions to stably maintain active expression states of homeobox genes in growing tissues. The protein encoded by ALL1 participates in a megadalton-size multiprotein complex that has chromatin remodelling, histone acetylation/deacetylation and histone methylation activities\textsuperscript{151}.

A suggested candidate for a mechanism of methylation modification was the DNA methyltransferase Dnmt1, which was originally reported to be overexpressed in cancer cells\textsuperscript{152}, but these studies have been controversial\textsuperscript{153,154}, and a well-controlled real-time PCR analysis did not show a significant increase\textsuperscript{155}. An alternative hypothesis involves expression of transcriptional repressors or, equivalently, the loss of activators, as the primary event. A recent example, put forward by Pelicci and colleagues to support the first possibility, is the recruitment of Dnmt1 and Dnmt3a to the retinoic-acid receptor (Rarb2) locus by binding of these methyltransferases to the oncogenic PML–RAR\textsubscript{α} fusion protein in promyelocytic leukaemia\textsuperscript{156}. This ‘secondary methylation’ scenario was questioned based on the finding of RARB2 methylation in many leukaemias that lack PML–RAR\textsubscript{α}\textsuperscript{157}, but these findings can be reconciled if other transcriptional repressors, present in the PML–RAR\textsubscript{α}–negative cases, also recruit methyltransferases. A second type of repressor that might shut down gene promoters before DNA methylation is typified by the SLUG transcription factor, which Fearon and colleagues found binds and silences the E-cadherin promoter — a known target of denovo methylation — in breast cancer cell lines\textsuperscript{158}, although, to our knowledge, there have not yet been reports of methyltransferase recruitment via SLUG.

In an important line of work that was initiated by the Goff laboratory in 1994 and substantially extended by Doug Dean’s laboratory, RB has been shown to function as a brake on the cell cycle at least in part by establishing and enforcing stable epigenetic silencing of its target genes. It does this by participating in a multiprotein complex that includes chromatin-remodelling enzymes of the SWI/SNF class\textsuperscript{159}, as well as histone deacetylases\textsuperscript{160,161} and epigenetic silencing proteins of the polycomb class (genetic antagonists of trithorax-group proteins)\textsuperscript{162}. Furthermore, based on at least two reports, the DNA methyltransferase Dnmt1 also binds to RB\textsuperscript{163,164}. Interestingly, the R12 gene, which encodes a histone methyltransferase that can associate with RB, was found by Huang and colleagues to sometimes be inactivated epigenetically and act as a tumour suppressor in mice\textsuperscript{165}. This finding indicates a hypothetical sequence of events in which a cancer-associated epigenetic lesion (including silencing at the R12 locus) exerts downstream effects that abrogate the function of a tumour-suppressor protein (RB), and this, in turn, erases stable epigenetic silencing at the cyclin E gene and other RB targets. Involvement of polycomb-group proteins in human cancer is not restricted to their interaction with RB: overexpression of Ezh2 in metastatic prostate cancer, already mentioned above, is another example\textsuperscript{166}.

**Epigenetic chemotherapy**

The demethylating drug 5-azaCdr — which inactivates methyltransferases — was first shown to transform cultured cells by Weinstein and colleagues in 1984 (Ref. 166), and these studies were stimulated by the earlier finding by Taylor and Jones that 5-azaCdr has reproducible effects on cell...
differentiation in tissue culture. This drug is now used in some clinical situations, notably as part of combined chemotherapy regimens for myelodysplastic syndrome and leukaemias. Part of its activity in patients might be due to its ability to reactivate growth suppressors, such as the INK4B (also known as p15) cyclin-dependent kinase inhibitor. However, data that were obtained with Dmnt1-knockout cells that are resistant to 5-AzaCdr indicate that the incorporation of the drug into DNA, and the resulting formation of covalent DNA--Dmnt adducts, might contribute to its cytotoxic effects. A necessity for intravenous administration has limited the usefulness of 5-AzaCdr, but an orally active inhibitor, zebularine, is entering clinical trials. 5-AzaCdr can also restore a normal pattern of imprinting to cells. The histone deacetylase inhibitor trichostatin A is already in use and seems to have efficacy against leukaemias. Potential synergy between trichostatin A and 5-azaCdr is now being tested in this clinical setting.

These promising therapies seek to reactivate tumour-suppressor genes that have been silenced epigenetically, and they are justified in patients with cancer in which other treatments have failed or are expected to fail. Nevertheless, a recent exchange in Science has highlighted the concern that genomic instability, because of hypomethylation, might be an adverse long-term consequence.

Conclusions

In the past 20 years, cancer epigenetics has come full circle, with a renaissance of interest in hypomethylation and its role in activating oncogenes and chromosomal rearrangement, as well as hypermethylation affecting tumour-suppressor genes. In the past 10 years, the discovery of imprinted genes and their role in cancer has added a new dimension to the field, and the impact of the role of chromatin modifications is just beginning to be felt. One of the most intriguing recent advances is the convergence of mechanistic studies linking DNA methylation, genomic imprinting and histone modification. Although cancer epigenetics is now considered to be well within the mainstream, there are several remaining questions that continue to limit its complete acceptance and still stimulate debate in assigning causal relationships. First, the mechanism of epigenetic inheritance other than DNA methylation is still largely unknown, yet it must be important as nonmethylated species handle epigenetic modification quite well. One could argue that the most important open question in molecular genetics is the mode of propagation of the histone code, through disassembly and reassembly during cell division, and the mechanism for this process. Cancer epigenetics will probably advance substantially when that process is better understood. Second, it is remarkable that tumour-suppressor genes are drawn from so many aspects of cell biology except DNA methylation. This indicates that the methylation changes themselves are secondary to other important causal elements, although that need not necessarily be the case. It might simply be that the known mediators of DNA methylation are factors that are essential for mammalian life, but that accessory factors for methylation propagation that are mutated are not known or that their role in methylation is not known. Third, the most compelling evidence for a causal role of epigenetic changes comes from the study of well-defined human genetic and epithelial syndromes. The main problem is that so few familial disorders seem to involve the epigenetic machinery. One notable exception is BWS, which is caused by epigenetic defects, and those alterations are specifically linked to cancer risk in affected patients. The future might reveal population epigenetic polymorphisms that contribute to cancer risk, but that do not cause a stak definable syndrome; the recent identification of a methylation variant that is linked to colorectal cancer might be such a polymorphism.

Finally, we would argue that age is central to our understanding of cancer epigenetics, an idea that goes all the way back to Holliday's observations of methylation erosion during ageing. The single leading risk factor for cancer is age. Although that has often been attributed to the accumulation of mutations over time, an alternative and complementary interpretation is that age itself disrupts the epigenetic programme, increasing cancer risk. This relationship might be even more true for non-malignant disease than for cancer, as epigenetics might explain why most common disorders that involve complex genetics are of adult onset; after all, the genome has been there since birth, so the genetic factors are presumably there as well. We feel that substantially more attention must be paid to epigenetic variation in the population, epigenetic changes during ageing and the relationship between these changes and common diseases including cancer. For these studies, the best model organisms are humans themselves.

40. Saito, Y.

42. Baylin, S. B.

47. Gonzalez-Zulueta, M.


61. Ehrlich, M.

62. Veigl, M. L.

64.什么呢? 请检查你的问题。


116. Bell, A. C., West, A. G. & Felsenfeld, G. The protein CTCF


116. Bell, A. C., West, A. G. & Felsenfeld, G. The protein CTCF


116. Bell, A. C., West, A. G. & Felsenfeld, G. The protein CTCF
Hereditary non-polyposis colorectal cancer is almost always associated with microsatellite instability, so what is the best way to identify the disorder at an early stage, and what should the next step be in preventing the development of colorectal cancer? Different clinical and molecular diagnostic guidelines have recently been proposed in the context of recent scientific advances, but how are these criteria interpreted and modified across the world?

Hereditary non-polyposis colorectal cancer (HNPCC) was originally called ‘cancer family syndrome’, as this autosomal dominant disease predisposes carriers of mutation to the development of several tumour types. In 1913, the pathologist Alfred Warthin published the first known case report of a family with characteristics of HNPCC. Clinical clues in similar families accumulated for many years and it was clearly delineated as a hereditary cancer syndrome that was distinct from familial adenomatous polyposis — which is caused by an inherited mutation of the tumour-suppressor gene APC — in the mid-1960s by Henry T. Lynch. For this reason, it is also known as Lynch syndrome. HNPCC has an incidence of 1:1,000 in the general population and up to 1:100 in individuals with colorectal cancer, which accounts for 1–5% of colorectal cancer cases3–5. It is characterized by an 80% lifetime risk for colorectal cancer and a 60% lifetime risk for endometrial cancer. It is important to emphasize that the lifetime risk of developing endometrial cancer in affected women is higher than their lifetime risk of developing colorectal cancer, so ‘Lynch syndrome’ might be a better choice of name than HNPCC6. Incidentally, individuals with HNPCC have also been shown to have an increased risk of developing extracolonic cancers, including those of the stomach, ovaries, small bowel, biliary tract, urethelium, kidney and central nervous system. Individuals with HNPCC colon cancers differ from those with sporadic colorectal cancer in several ways: the tumours are diagnosed at an earlier age; they are frequently located in the proximal colon (60–70%); they have an increased risk of developing synchronous or metachronous colon cancers; and they have a better prognosis7–10. Advances in the understanding of the genetic basis of HNPCC carcinogenesis have led to efforts to exploit this knowledge clinically, primarily in the form of predictive diagnostic criteria. In conjunction with family history, molecular tests have been designed to improve cancer-risk assessment of individuals. Diagnostic guidelines for HNPCC that were used previously were somewhat confusing and could now be outdated, as the advances in our understanding of the disease have progressed substantially over the past decade. Here, we discuss diagnostic guidelines for HNPCC across the world; these have been developed over time and several modifications have been made. At an international workshop that was held recently in Bethesda, Maryland, in the United States11, we discussed the recent advances in the understanding of the genetic basis of HNPCC and the Bethesda guidelines were revised. These guidelines are intended to be used to make a decision as to whether genetic testing of individuals should be performed in an attempt to detect HNPCC early. The early knowledge that an individual carries a defective allele of one of the DNA mismatch-repair (MMR) genes could allow individuals to realize preventive measures to delay and/or reduce the chance of getting the malignant disease.

**Online links**

**DATABASES**

The following terms in this article are linked online to:

- CancerGene: http://cancergene.gov
- acutelymphoblastic leukemia | acute myelogenous leukemia | cervical cancer | colon cancer | leukaemia | pancreatic cancer | stomach cancer | uterine cancer | Wilms tumour

**FURTHER INFORMATION**

DNA Methylation Society: http://dnamethsosc.server101.com/

Imprinted Gene Catalogue: http://cancer.otago.ac.nz/IGC/Web/home.html

Imprinting: http://www.geneprint.com

Access to this interactive links box is free online.

**MMR defects cause HNPCC**

HNPCC11,12 is caused by germline mutations in any one of five DNA MMR genes — MSH2, M L1, M SH6, infrequently PM S2 and, rarely, PM S1 (refs 12–16; FIG. 1; BOX 1). Genetic testing for HNPCC is therefore defined as the determination of the primary DNA sequence of MSH2, ML1 or M SH6 to detect heritable disease-related genotypes or mutations for clinical purposes. Defective MMR leads to an inability to repair base–base mismatches and small insertions and deletions, so causing an increased genomic mutation rate, which can lead to cancer. Paradoxically, however, as many as 50% of the suspected cases of HNPCC are not confirmed by a genetic defect (that is, mutation in one of the known MMR genes), so it remains a key issue to define the genotype–phenotype relationship between these and confirmed cases17,18. Epigenetic silencing of M L1 is also common in non-hereditary cancers that resemble HNPCC19, which further confuses diagnosis of HNPCC.

Microsatellite DNA comprises repetitive sequences of 1–6 bases that are scattered throughout the human genome — most commonly as (CA)n. The replication machinery slips more frequently on repetitive sequences than on non-repetitive sequences; so microsatellite sequences accu-