Phenotypic plasticity and the epigenetics of human disease

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It is becoming clear that epigenetic changes are involved in human disease as well as during normal development. A unifying theme of disease epigenetics is defects in phenotypic plasticity — cells' ability to change their behaviour in response to internal or external environmental cues. This model proposes that hereditary disorders of the epigenetic apparatus lead to developmental defects, that cancer epigenetics involves disruption of the stem-cell programme, and that common diseases with late-onset phenotypes involve interactions between the epigenome, the genome and the environment. Increased understanding of epigenetic-disease mechanisms could lead to disease-risk stratification for targeted intervention and to targeted therapies.

The original definition of epigenetics by Waddington in 1942 (ref. 1) — the idea that that phenotype arises from genotype through programmed change — is now what is considered to be developmental biology. The modern definition of epigenetics is information heritable during cell division other than the DNA sequence itself. It is becoming increasingly clear that there is great overlap between these two definitions — developmental processes are regulated largely by epigenetics, because different cell types maintain their fate during cell division even though their DNA sequences are essentially the same.

What is epigenetic disease? Genetic lesions — sequence changes, breakages and deletions — can be easily visualized, but what about epigenetic lesions? Several defects in the epigenome are known to lead to disease (Fig. 1), including changes in the localized or global density of DNA methylation, and incorrect histone modification. Other defects that might cause disease involve altered distribution or function of chromatin-modifying proteins that, in turn, leads to aberrant gene expression. Another intriguing possibility is the disruption of higher-order loop structure in disease (Fig. 1).

Studies of monogenic disorders involving imprinted genes or the epigenetic machinery have revealed a great deal about the nature of the *cis*-acting regulatory marks and *trans*-acting factors that modulate the epigenome. Two decades ago, cancer epigenetics was viewed with some scepticism, but it is now widely accepted. However, important questions about the mechanism, timing and consequences of epigenetic disruption remain. Whether other common diseases have an epigenetic basis is still open to speculation, but if they do, this holds great promise for medicine.

Here I review the epigenetics of single-gene disorders, cancer and common complex diseases. I suggest that a common theme to disease epigenetics is the disruption of phenotypic plasticity — the ability of cells to change their behaviour in response to internal or external environmental cues — an idea that resonates with Waddington's original definition of epigenetics. I also discuss therapeutic implications of disease epigenetics.

Imprinted gene disorders

There are two classes of monogenic epigenetic disease: those involving genes that are regulated epigenetically, such as imprinted genes, and those that affect the epigenome as a whole, such as modifiers of DNA methylation.

Epigenetic disease genes

An example of the first class of monogenic epigenetic disease is Beckwith–Wiedemann syndrome, which is characterized by prenatal overgrowth, a midline abdominal wall and other malformations, and cancer. Studies of patients with Beckwith–Wiedemann syndrome have taught us a great deal about the mechanisms of normal imprinting. Patients with Beckwith–Wiedemann syndrome show disrupted imprinting of either or both of two neighbouring imprinted subdomains on 11p15, revealing clustering of imprinted genes (Fig. 2). The first is the *H19*/*IGF2* (imprinted, maternally expressed, untranslated mRNA/insulin-like growth factor 2) imprinted subdomain, which is regulated by a differentially methylated region (DMR) that is methylated on the paternal but not the maternal allele. The second subdomain includes $p57^{KIP2}$ (a cyclin-dependent kinase inhibitor), *TSSC3* (a pleckstrin homology-like domain), *SLC22A1* (an organic cation transporter), K_vLQT1 (a voltage-gated potassium channel) and *LIT1* (*KCNQ1* overlapping transcript 1), with the subdomain regulated by a second DMR, upstream of *LIT1*, that is normally methylated on the maternal but not the paternal allele (see ref. 2 for a review). In patients with Beckwith–Wiedemann syndrome, microdeletions within each imprinted subdomain have confirmed the regulatory role of these sequences, because individuals with these deletions show loss of normal imprinted gene regulation[3,4] (Fig. 2). Some patients with Beckwith–Wiedemann syndrome show loss of imprinting of *IGF2*, which leads to a double dose of this autocrine factor, resulting in tissue overgrowth and increased cancer risk[5]. The mechanism involves aberrant methylation of the maternal *H19* DMR (Fig. 2). Other patients with this syndrome show localized abnormalities of allele-specific chromatin modification[6] affecting $p57^{KIP2}$, a cyclin-dependent kinase inhibitor (Fig. 2). Thus, Beckwith–Wiedemann syndrome illustrates hierarchical organization of epigenetic regulation in progressively larger domains.

A pair of imprinted-gene disorders that are associated with mental retardation — Prader–Willi syndrome and Angelman syndrome — involve adjacent reciprocally imprinted genes, *SNRPN* (small nuclear ribonucleoprotein polypeptide N) and *UBE3A* (ubiquitin–protein ligase E3A), on chromosome 15. Microdeletions in patients with both Prader–Willi syndrome and Angelman syndrome reveal the location of the domain that regulates imprinting of both genes[7].

Another pair of human disorders caused by different alterations at the same locus are Albright hereditary osteodystrophy and

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pseudohypoparathyroidism type IA (PHPIA). Albright hereditary osteodystrophy is characterized by short stature and ectopic calcifications, and caused by mutational inactivation of guanine nucleotide regulatory protein (encoded by *GNAS1*). PHPIA is a more severe phenotype of multiple hormone resistance caused by tissue-specific differential imprinting of splice variants of the same gene[8]. It is unlikely that this complex pattern of imprinting would have been understood without concomitant clinical studies.

Single-gene disorders of the epigenetic machinery

The other class of monogenic epigenetic disease involves genes that encode components of the machinery that regulates the epigenome. Mutation of these genes causes developmental disorders. For example, Rett syndrome involves mutations of the methyl CpG-binding protein 2 (*MeCP2*) gene, which encodes a protein that binds to methylated DNA sequences[9]. In Rett syndrome, DNA methylation proceeds normally but epigenetic silencing is impaired because of a failure to properly recognize this mark[10] (Fig. 3). What is striking about the phenotype of this disorder is that prenatal and early infant development is normal, and erosion of neurodevelopmental milestones is not seen until later childhood.

Epigenetically disrupted development can occur in various biological pathways or systems. Immunodeficiency/centromeric instability/facial anomalies (ICF) syndrome, for example, affects the immune system and involves mutations of the DNA methyltransferase gene *DNMT3B*, which is responsible for *de novo* DNA methylation during development[11]. Patients wih ICF syndrome show failure of normal immune system development as well as developmental dysmorphology, which could involve failure of heterochromatin formation and might result from *DNMT3B* having a role in immunoglobulin gene silencing and reactivation[12].

A striking example of developmental disruption caused by mutations in a chromatin factor gene is alpha-thalassaemia/mental retardation, X-linked (ATRX) syndrome, the gene for which is a helicase involved in chromatin remodelling. Mutations lead to defects in psychomotor, urogenital and haematopoietic development, with maturational defects in erythroid precursors resembling those of alpha-thalassaemia[13]. Rubinstein–Taybi syndrome involves the CREB (cyclic-AMP responsive-element-binding protein)-binding protein CBP, which has histone acetyltransferase activity, and mutations in *CBP* lead to skeletal and cardiac malformations, as well as neurodevelopmental malformations and loss of neural plasticity[14]. A common theme of these disorders is that mutations in epigenome regulators cause developmental disruption and often cause phenotypic changes in multiple organ systems.

DNA methylation in cancer

Cancer is commonly characterized as showing global hypomethylation and site-specific gene hypermethylation, but a more accurate description is that cancer involves both global and gene-specific hypomethylation and hypermethylation, as well as widespread chromatin modifications (Fig. 3). The first epigenetic change described in tumours was gene hypomethylation[15], and we now know that many growth-promoting genes are activated through hypomethylation in tumours, including *HRAS*, cyclin D2 and maspin in gastric cancer, carbonic anhydrase IX in

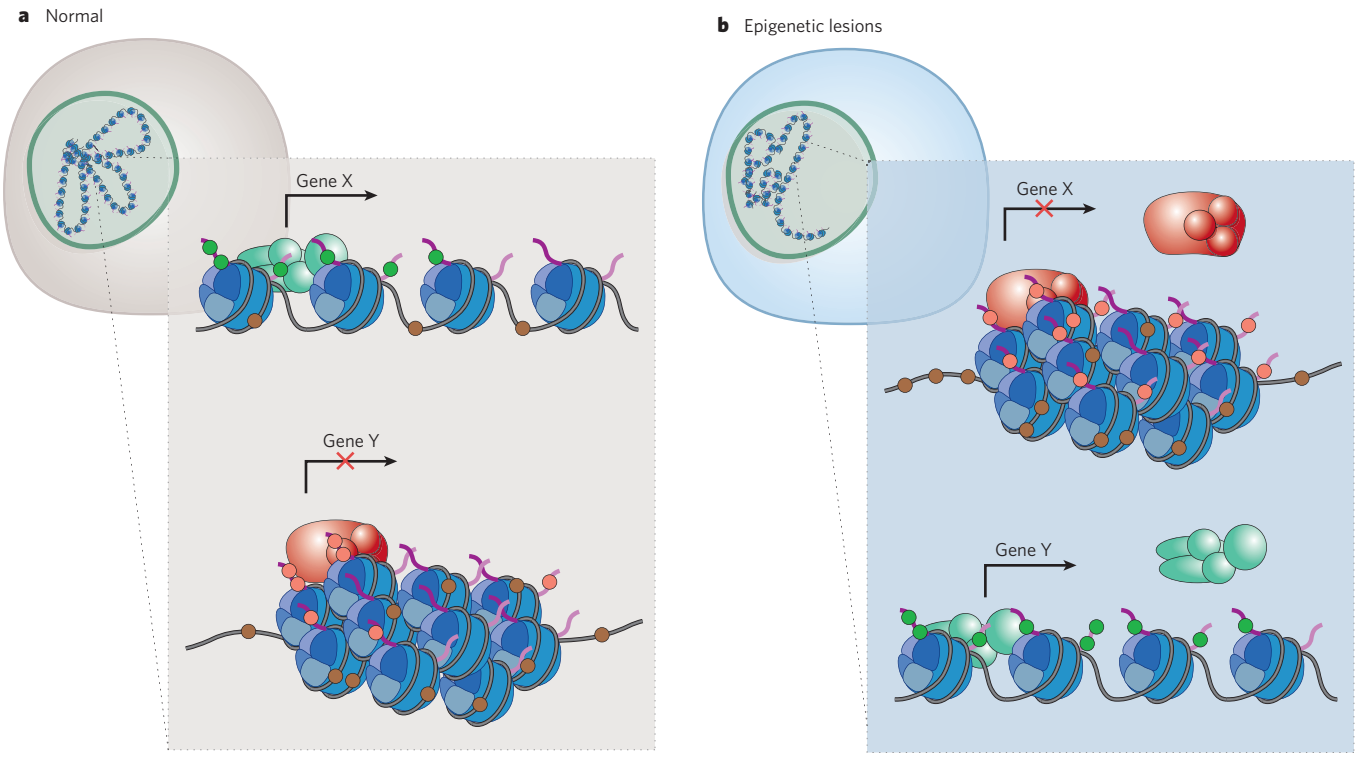

Figure 1 | The nature of epigenetic lesions. Although the nature of genetic lesions is well understood, epigenetic lesions have been more difficult to define. Here we depict known and possible defects in the epigenome that could lead to disease. **a**, X is a transcriptionally active gene with sparse DNA methylation (brown circles), an open chromatin structure, interaction with euchromatin proteins (green protein complex) and histone modifications such as H3K9 acetylation and H3K4 methylation (green circles). Y is a transcriptionally silent gene with dense DNA methylation, a closed chromatin structure, interaction with heterochromatin proteins (red protein complex) and histone modifications such as H3K27 methylation (pink circles). **b**, The abnormal cell could switch its epigenotype through the silencing of normally active genes or activation of normally silent genes, with the attendant changes in DNA methylation, histone modification and chromatin proteins. In addition, the epigenetic lesion could include a change in the number or density of heterochromatin proteins in gene X (such as EZH2 in cancer) or euchromatic proteins in gene Y (such as trithorax in leukaemia). There may also be an abnormally dense pattern of methylation in gene promoters (shown in gene X), and an overall reduction in DNA methylation (shown in gene Y) in cancer. The insets show that the higher-order loop configuration may be altered, although such structures are currently only beginning to be understood.

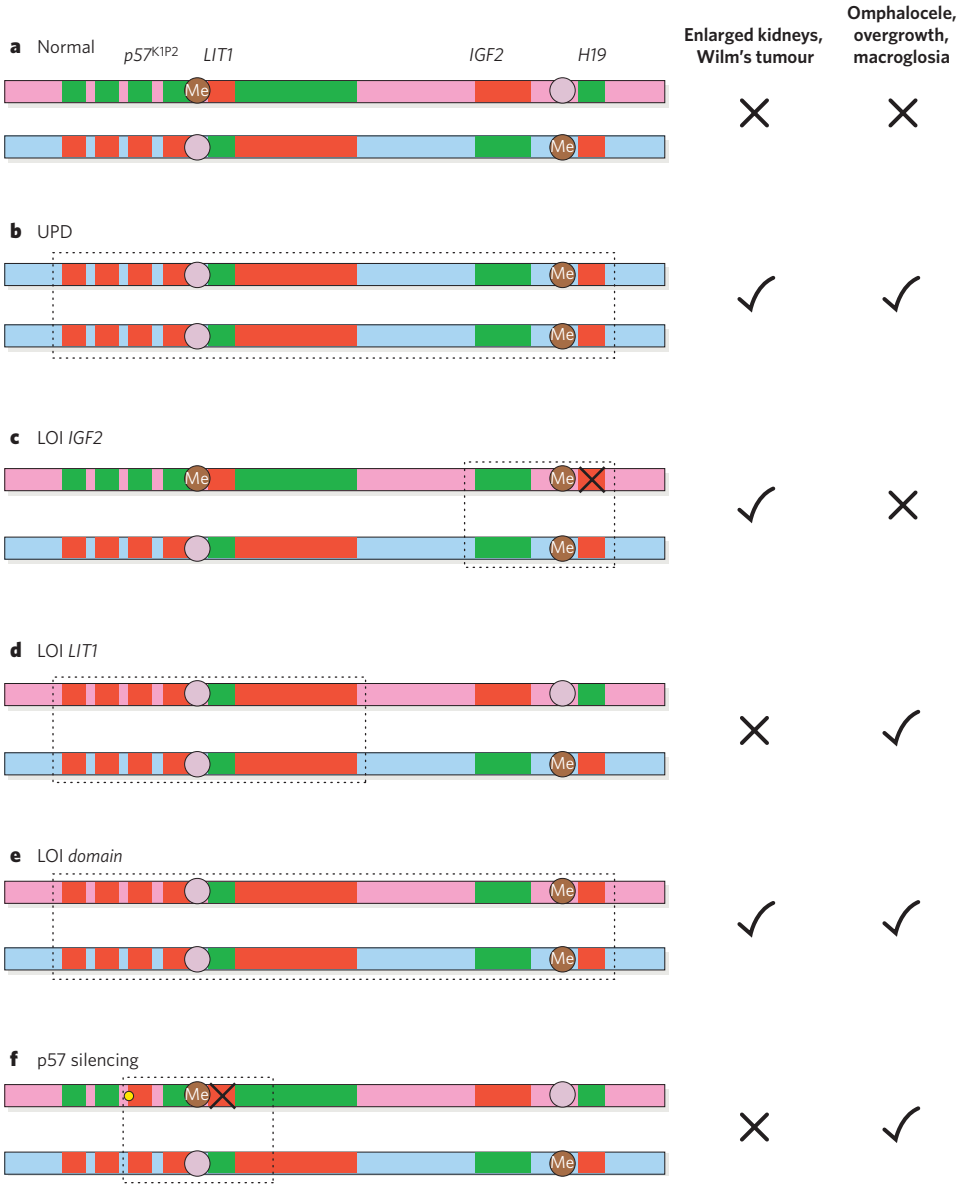

Figure 2 | Beckwith–Wiedemann syndrome as an example of a monogenic disease that reveals mechanisms of normal epigenetic regulation. Depicted are a pair of normal chromosomes (**a**) and several illustrative lesions (**b**–**f**); maternal chromosomes are pink and paternal blue, and DMRs are indicated (Me). Imprinted genes are depicted, not to scale or in their entirety, with green representing active and red representing silent alleles, respectively. Patients with uniparental disomy (UPD, **b**) have complete genetic replacement of the maternal allele region with a second paternal copy (dashed enclosure). Loss of imprinting (LOI) of *IGF2* (**c**) causes a switch in epigenotype of the *IGF2/H19* subdomain (dashed enclosure). LOI of *LIT1* (**d**) causes a switch in epigenotype of the *p57/K$_V$LQT1/LIT1* subdomain. Some patients show LOI of the entire imprinted gene domain in the absence of UPD (**e**). Other patients show localized chromatin disruption (small yellow circle, **f**) silencing *p57*^{KIP2}. Thus, imprinting is organized hierarchically into a large domain containing two smaller subdomains. In addition, some patients show microdeletions in either of the two domains (black crosses), revealing the location of imprinting control centres. The domain organization similarly reveals the contiguous gene syndrome nature of the disease. Patients with involvement (genetic or epigenetic) of the *IGF2/H19* domain have enlarged kidneys and Wilms' tumours. Patients with involvement of the *p57/K$_V$LQT1/LIT1* domain show somatic overgrowth, an enlarged tongue and omphalocele (in which abdominal organs protrude from the navel). And children with involvement of both domains show both phenotypes.

renal-cell cancer, and S100 calcium-binding protein A4 in colon cancer (see refs 16, 17 for reviews). In addition, many C/T (cancer/testis) genes that are expressed normally in the healthy testis are activated in other cells by hypomethylation in cancer, including the melanoma-associated antigen (*MAGE*) gene family, which has antigenic and immunotherapeutic value in melanoma and glioblastoma[16,17] and the oncogenic micro RNA let-7a-3 (ref. 18). Activation of the human papilloma virus HPV16 by hypomethylation is a major mechanism affecting tumour latency in cervical cancer[16,17]. Recently, oestrogen- and tamoxifen-induced activation of *PAX2* and endometrial proliferation, leading to cell proliferation, was found to be cancer-specific because of *PAX2* promoter hypomethylation in the tumours[19].

By contrast, tumour suppressor gene silencing has been linked to promoter hypermethylation, first described for *RB*, the gene associated with retinoblastoma[20], and many other tumour suppressor genes, including *p16*, *VHL* (von Hippel–Lindau), *MLH1*, *APC* (adenomatosis polyposis coli) and E-cadherin (see ref. 21 for a review). Recent high-throughput approaches have been used to identify other candidate genes[22,23]. An exciting demonstration of domain-wide silencing involves an entire chromosomal band[24], suggesting a disturbance of higher-order chromatin (Fig. 1). However, studies focused on loss of DNA methylation in cancer may have overlooked hypomethylation of tissue-specific methylation marks at CpG islands[25]. Indeed, whole-genome analysis suggests that CpG island hypermethylation may be less widespread than had been suspected[26]. It should also be noted that as many genes are silenced as are activated in tumours by both drug-induced hypomethylation and by knockdown of DNA methyltransferases[27], thus both hypomethylation and hypermethylation can lead to gene activation and gene silencing in cancer.

Loss of imprinting in cancer

The earliest clue that genomic imprinting might be involved in cancer came from two rare types of tumour: hydatidiform moles, which are malignant trophoblastic tumours caused by a pregnancy arising from two complete sets of the paternal genome, and ovarian teratomas, which are benign tumours with many tissue types that arise from two complete sets of the maternal genome. Molecular evidence for a role of genomic imprinting in cancer emerged from studies showing a universal loss of the maternal allele in Wilms' tumours and embryonal rhabdomyosarcoma, with loss of heterozygosity (LOH) of 11p15, implying that normally only the maternal allele of an as yet unidentified tumour suppressor gene might be expressed[28]. So it was surprising that the first molecular evidence for a role of genomic imprinting in cancer was loss of imprinting (LOI), causing abnormal activation of the normally silent copy of *IGF2*, an important autocrine growth factor, leading to pathological biallelic expression of *IGF2* in Wilms' tumours, the most common childhood solid tumour[29,30]. LOI refers either to aberrant activation of the

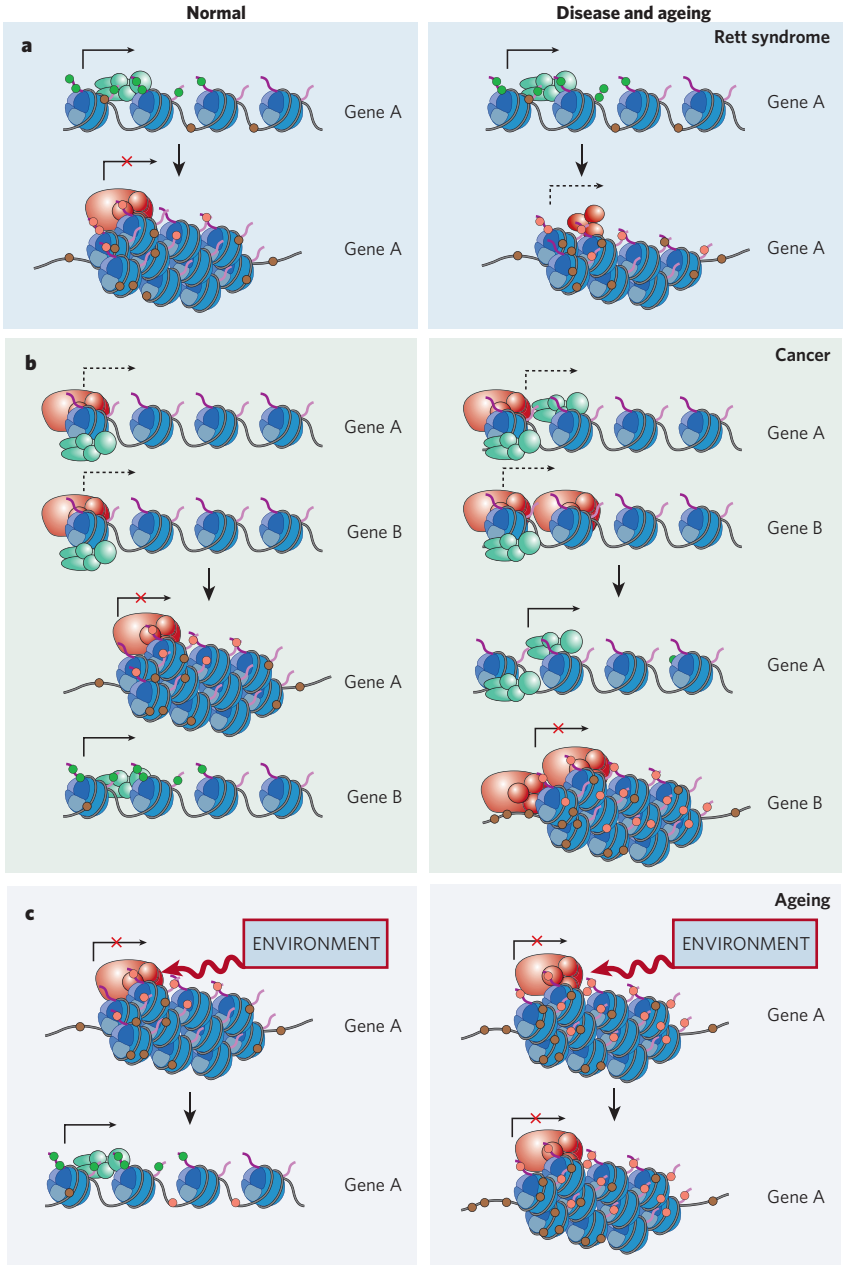

Figure 3 | Phenotypic plasticity and the epigenetics of human disease and ageing. A common feature of epigenetic lesions in human disease is that they affect a cell's ability to change its phenotype. **a**, In monogenic disorders such as Rett syndrome, a defect in the normal epigenetic apparatus itself impedes normal development. DNA methylation (brown circles on the DNA) proceeds normally but is not recognized owing to the absence of the MeCP2-methylation-interacting protein (large red oval). This leads to failure to completely silence genes appropriately during development (dashed arrow). **b**, Cancer involves many epigenetic lesions that could affect a pluripotent programme in tissue-specific stem cells, possibly leading to an incorrect distribution of differentiated cell lineages (indicated by the bivalent euchromatin and heterochromatin proteins shown in the upper left panel) and normal tissue-specific silencing of gene A and activation of gene B after differentiation (lower left panel). Examples of epigenetic lesions found in cancer include changes in chromatin proteins in stem cells caused by increased expression of *MLL1* in leukaemia (upper right panel, green complex above gene A representing *HOX* genes), leading to aberrant *HOX* expression in differentiated cancer lineages (lower right panel). Another epigenetic lesion found in cancer is increased expression of *EZH2* (upper right panel, red complex above gene B, representing diverse tumour suppressor genes), leading to aberrant silencing of these genes in differentiated cancer lineages (lower right panel). **c**, Ageing involves a loss of the normal plasticity of response to internal and external environmental signals. The epigenome could have an important role in ageing if the aged epigenome is less responsive to such signals. For example, a gene (at this point hypothetically) showing increased H3K9-methylation (upper right panel, red circles on nucleosomes) or DNA methylation (brown circles on DNA), might be relatively refractory to environmentally induced activation (lower right panel) than if the gene had not undergone age-dependent epigenetic change (left panels).

normally silent allele of an imprinted growth-promoting gene, or aberrant silencing of the normally expressed copy of an imprinted tumour suppressor gene, such as the still unidentified locus on 11p15 (ref. 31). Subsequently, LOI of *IGF2* has been found to be common in lung cancer[32], breast cancer[33], ovarian cancer[34] and glioma[35]. LOI of other genes include *ARHI* in breast cancer[36], *DLK1/GTL2* in pheochromocytoma, neuroblastoma and Wilms' tumour[37], and *PEG1* (also known as *MEST*) in breast cancer[38].

Chromatin and cancer

It has become increasingly clear that in cancer chromatin modifications are at least as widespread and important as alterations in DNA methylation. For example, overexpression of the polycomb group protein EZH2, a H3 lysine-27 (H3K27) histone methyltransferase, is found in metastatic prostate cancer and may lead to widespread transcriptional repression[39] (Fig. 3). Generalized loss of H4 acetylated Lys-16 (H4K16ac) and trimethylated Lys-20 (H4K20me3) is found in lymphoma and colorectal cancer, which could also lead to transcriptional silencing[40] (Fig. 3). It is not surprising that both DNA methylation and histone modification are altered in cancer, given their interdependence in normal development. For example, in the fungus *Neurospora* DNA methylation depends on H3K9 methylation[41], and in mice DNA methylation of homeobox (*Hox*) genes depends on a full length *Mll* (myeloid/lymphoid or mixed-lineage leukaemia) gene[42]. DNMT1 interacts with the H3K9 methyltransferases G9a and SUV39H1, which are needed for normal replication-dependent DNA methylation[43]. Some chromosomal rearrangements and, less commonly, mutations in cancer act by causing widespread chromatin disruption. *MLL1*, which is rearranged and activated in acute lymphoblastic leukaemia, methylates H3K4 to activate gene expression and interacts with integrase integrator 1 (*INI1*) in the SWI/SNF chromatin remodelling complex[44]. *INI1* is mutated in rhabdoid tumour, a deadly soft-tissue malignancy[45]. Sotos syndrome, which is characterized by tissue overgrowth, leukaemia and Wilms' tumour, is caused by mutations in *NSD1*, an H3K36/H4K20 methyltransferase[46]. Thus, a strong argument can be made for chromatin modifications driving epigenetic disruptions during cancer development.

The argument for causality

One problem with the idea that alterations in DNA methylation underlie cancer is that no mutations in either the methylation modification or

the recognition machinery have yet been identified in human cancer. Indeed, congenital disorders involving such modifications — for example, Rett syndrome (*MeCP2*) and ICF syndrome (*DNMT3B*) — do not carry an increased cancer risk, in contrast to the chromatin-modifying disruptions described above. In addition, epigenetic changes might be a consequence of altered gene expression rather than causal; it has been known since the 1980s that numerous genes are aberrantly expressed in tumours[47]. Furthermore, activation of tumour suppressor genes by 5-aza-2′-deoxycytidine or *DNMT1* knockout may not be stable, as has been shown for both *MLH1* (ref. 48) and *p16* (ref. 49), suggesting that the altered methylation might be a consequence rather than a cause of gene silencing.

So how can a convincing causal argument be made? Good evidence would be constitutional epigenetic alterations linked to cancer risk. The first such example was Beckwith–Wiedemann syndrome, which leads to an 800-fold increased risk of embryonal tumours — that is, those involving residual fetal tissues, such as Wilms' tumour of the kidney and rhabdomyosarcoma[50]. LOI of *IGF2* is specifically associated with increased cancer risk in children with Beckwith–Wiedemann syndrome, even though it occurs in only a fraction of the affected individuals[5] (Fig. 2). Thus, the epigenetic change precedes cancer and confers risk for cancer, a strong argument for causality. LOI of *IGF2* was found in adults, at a frequency of 5–10%[51], and is associated with a fivefold increased frequency of benign and malignant colorectal neoplasms (and 20-fold for cancer), as well as an increased family history of cancer, consistent with a causal role in cancer predisposition[52,53]. Another example of epigenetic alterations in normal tissue is the hypermethylation of *p16* that occurs with ageing[54] and in the normal tissue of women with breast cancer[55], although neither case has yet been linked to cancer risk.

Experimental data in mice further support a causal role for epigenetic changes in cancer. When *DNMT1* hypomorphs are crossed with Min (multiple intestinal neoplasia) mice with an *Apc* mutation, they show an increased frequency of intestinal neoplasia and liver cancers[56]. Hypomethylation also causes increased chromosomal instability, leading to aggressive T-cell lymphomas[57], as well as an increase in sarcomas in mice with *p53* and neurofibromin 1 (*NF1*) mutations[58]. DNA hypermethylation is also important, as *DNMT1* hypomorphs also show delayed progression of adenomas in Min mice[56,59], suggesting that hypomethylation is more important in the earliest stages of carcinogenesis, whereas hypermethylation has a greater role during tumour progression. In addition, engineered loss of one allele of *HIC1* leads to an increased number of late-onset tumours with epigenetic silencing of the remaining allele[60]. Genetically induced LOI of *IGF2* increases the frequency of adenomas in mice caused by mutations in *Apc*[61]. Moreover, engineered global LOI leads to intestinal and hepatic tumours in chimaeric mice[62].

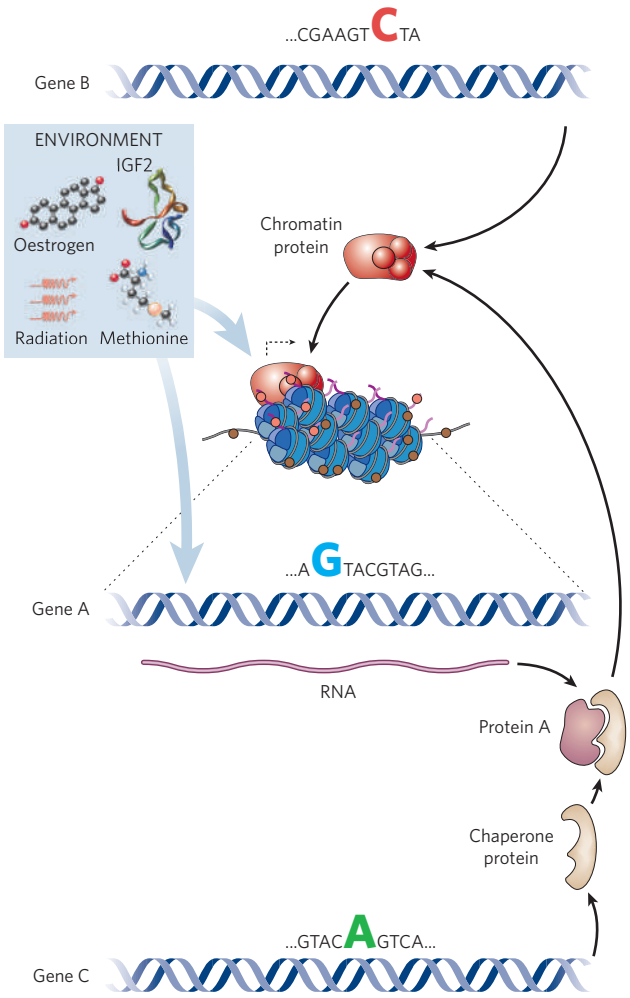

Figure 4 | The epigenome at the intersection between environment and genetic variation. According to the common disease genetic and epigenetic (CDGE) hypothesis, the epigenome may modulate the effect of genetic variation (example shown is the large nucleotide in gene A, which could be C or G), either by affecting the gene's expression through the action of chromatin proteins or DNA methylation, or by modulating protein folding of the gene product of the variant locus or chromatin protein. The epigenome may, in turn, be affected by sequence variation in the genes encoding chromatin or chaperone proteins (genes B and C, respectively). Environmental factors (such as toxins, growth factors, dietary methyl donors and hormones) can affect the genome and the epigenome.

Cancer epigenetics and the stem-cell hypothesis

Although epigenetic alterations are commonly looked on as surrogates for genetic change in cancer, they are probably also critical first steps in neoplastic progression, disrupting the normal stem- or progenitor-cell programme, for example by stimulating stem-cell proliferation outside their normal microenvironment[63]. This 'epigenetic progenitor model', in which cancer originates in stem or progenitor cells after epigenetic alterations, is supported by the ubiquitous early nature of epigenetic changes in cancer, discussed above, as well as the demonstration of altered progenitor cells in normal tissues of patients with cancer.

LOI of *IGF2* leads to an expanded progenitor-cell compartment in the intestine of mice harbouring an *Apc* mutation, and increased expression of progenitor-cell markers[61,64], a feature also seen in humans with LOI of *IGF2* and increased risk of colon cancer[61]. Similarly, LOI of *IGF2* in Beckwith–Wiedemann syndrome is specifically associated with cancer risk and leads to the expansion of nephrogenic progenitor cells[65]. Further support for the model comes from the fact that mouse melanoma and medulloblastoma nuclei can be cloned to form blastocysts or chimaeric mice[66]. Although mice derived from the former show an increased propensity to form melanomas, many of the tumour properties must be epigenetic in origin and some cells within the tumour are pluripotent[66].

In breast cancer, widespread epigenetic alterations are found in tumour cells, stromal cells and the myoepithelium, suggesting that the entire tumour microenvironment, including apparently normal cells, is the target of epigenetic disruption[67]. Cancers also seem to show increased epigenetic plasticity. This epigenetic plasticity may be an inherent property of the stem cells from which cancers arise, for example the bivalent nature of adjacent H3K4 and H3K27 methylation that is seen at many genomic sites in stem cells but not in somatic cells after differentiation[68]. The gene *MLL1*, which is rearranged and activated in childhood leukaemias, is also a key regulator of normal stem-cell differentiation[69,70]. Polycomb group genes might also be tumour progenitors, as they are overexpressed in cancer, as noted earlier, and they repress developmental regulators in embryonic stem cells[71]. Thus, epigenetics seems to be central to plasticity both in development and in tumour cells, and epigenetic discovery will be critical to understanding these.

Epigenetics and common complex disease

The next great frontier in the epigenetics of human disease is to establish its potential role in common non-neoplastic human diseases. At the moment, the most appealing candidates are disorders affecting behaviour, on the basis of clues from Rett syndrome and Prader–Willi syndrome, as well as the intriguing story of Turner syndrome in girls with only one X chromosome. Girls lacking the paternal X chromosome exhibit behavioural socialization problems more frequently than girls lacking the maternal X chromosome[72], and a candidate imprinted gene region that could be responsible has been identified[73]. Autism and bipolar disorder are two common complex traits that have defied gene identification, and both show surprisingly high frequencies of phenotypic discordance in monozygotic twins. Only 60% concordance was reported in autism using strict criteria[74], and neuroanatomical differences have been found in cerebellar grey- and white-matter volumes between discordant monozygotic twins[75]. In bipolar disorder, 30% of monozygotic twins are discordant[76], and the disease itself is episodic, with patients being seriously ill at some times and perfectly normal at others, often for long stretches of time, for no apparent reason. Some studies of both autism and bipolar disorder have also shown parent-of-origin-specific linkage, with excess transmission of paternal alleles to autism cases[77], and excess transmission of maternal alleles to bipolar disorder cases[78].

A third candidate common disease with an epigenetic component is systemic autoimmune disease. Aberrant hypomethylation is found in T cells of patients with systemic lupus erythematosis, including in genes such as lymphocyte function-associated antigen-1, which is overexpressed in lupus T cells[79]. Treatment of viable T cells with 5-aza-2′-deoxycytidine induces a syndrome in mice similar to systemic lupus erythematosis[80]. Procainamide and hydralazine both cause hypomethylation and can cause lupus, and treatment of T cells with these drugs elicits a lupus-like syndrome in mice[81].

Epigenetics and the environment

The epigenome is an important target of environmental modification. Environmental toxins such as heavy metals disrupt DNA methylation and chromatin[82]. Oestrogenic and anti-androgenic toxins that decrease male fertility alter DNA methylation, and these changes are inherited by subsequent generations[83]. Dietary modification also can have a profound effect on DNA methylation and genomic imprinting. Deficiency in folate and methionine, necessary for normal biosynthesis of S-adenosylmethionine, the methyl donor for methylcytosine, leads to aberrant imprinting of IGF2 (ref. 84), and methylation supplementation can cause methylation and silencing of a retroposon in mice with silencing of the nearby agouti coat-colour gene[85]. Colorectal cancer risk is linked to both dietary folate deficiency and variants in methylenetetrahydrofolate reductase, which has a critical role in directing the folate pool toward remethylation of homocysteine to methionine[86]. A remarkable example of an environmental effect on the epigenome is the modification of glucocorticoid receptor methylation seen in the hippocampus of rat pups in response to maternal grooming[87]. A surprising environmental modulator of the epigenome is assisted reproductive technology (ART), which has been shown to be the method of conception at higher than expected frequency in Beckwith–Wiedemann syndrome and Angelman syndrome[88]. Intriguingly, all but 1 of the 14 reported cases of Beckwith–Wiedemann syndrome associated with ART involved hypomethylation of *LIT1* (ref. 89), although this abnormality is normally present in only about one-third of patients with Beckwith–Wiedemann syndrome.

The common disease genetic and epigenetic (CDGE) hypothesis argues that in addition to genetic variation, epigenetics provides an added layer of variation that might mediate the relationship between genotype and internal and external environmental factors[90]. This epigenetic component could help to explain the marked increase in common diseases with age, as well as the frequent discordance of diseases such as bipolar disorder between monozygotic twins[76]. A common characteristic of ageing is a time-dependent decline in responsiveness or adaptation to the environment, a form of loss of phenotypic plasticity. This loss of phenotypic plasticity could be mediated epigenetically if loss of the normal balance between gene-promoting and gene-silencing factors occurred across the genome (Fig. 3). This idea is supported by a study showing greater variance of total DNA methylation and histone H3K9 acetylation in older monozygotic twins than in younger twins, although that study did not measure epigenetic changes over time in the same individual[91].

The CDGE hypothesis is also supported by two compelling lines of evidence in model organisms. First, inhibition of the chaperone protein Hsp90 in *Drosophila* leads to the expression of previously latent heritable mutations within a single meiotic cell division, which then become independent of Hsp90 (ref. 92). This mutational suppression is chromatin mediated and can be reversed by mutations in several trithorax group proteins[93]. Second, a screen for genes that cooperate in disrupting *Caenorhabditis elegans* phenotypes revealed six 'hub' genes that interacted with as many as one-quarter of all genes tested. All were components of chromatin-modifying complexes[94]. Thus, common diseases may involve phenotypic variants with both genetic variation and environmentally triggered epigenetic change that modulates the effects of DNA sequence variation (Fig. 4). These epigenetic modifiers are, in turn, affected by variation in the genes that encode them, and environmental factors (hormones, growth factors, toxins and dietary methyl donors) influence both the genome and epigenome (Fig. 4). This idea can be tested by incorporating an assessment of the epigenome into population epidemiological studies (see ref. 95 for a review), rather than simply stratifying risk for environmental exposures as is done currently.

Prospects for epigenetic therapy

As epigenetic mechanisms for human disease are identified, epigenetic therapies are being developed or rediscovered. Some drugs are used specifically because of their known effects on the epigenome. For example, two classes of epigenome-modifying agent are currently in clinical trials for cancer, for example, for the treatment of myelodysplasia[96]: DNA methyltransferase inhibitors such as decitabine, and histone deacetylase inhibitors such as SAHA (suberoylanilide hydroxamic acid). SAHA is being used for cancer treatment, although its *in vivo* targets are still unknown. The overall response rate with decitabine in a phase III study showed a small but statistically significant difference for myelodysplasia (9% complete response and 8% partial response, compared with no response in controls), and half of the clinically responsive patients showed a cytogenetic response[97]. One cautionary note about the use of nonselective agents that inhibit DNA methylation is that these drugs may activate as many genes as they silence[27]. An effect opposite to that of methyltransferase and histone deacetylase inhibitors is achieved through rational drug design of histone acetyltransferase inhibitors, for example, bisubstrate analogues such as Lys-CoA, a selective *p300/CBP* inhibitor[98]. Such drugs may be useful in cancer treatment, because *p300*-negative cells undergo increased apoptosis after chemotherapy[99].

Some drugs that have an effect on the epigenome are already in widespread use, but their epigenetic effect has only recently been discovered. For example, valproic acid is used to treat various disorders, including seizures, bipolar disorder and cancer, and valproic acid was recently found to be a potent histone deacetylase inhibitor[100]. A relatively simple drug strategy could be to target rationally designed small compounds to a epigenetically altered pathway, rather than attempting to repair an epigenetic lesion. For example, in patients with LOI of *IGF2*, the IGF2 signalling receptor, IGF1R tyrosine kinase, or the downstream Akt or ERK signalling pathways could be targeted with existing drugs or compounds under development rather than attempting to reverse the epigenetic lesion itself.

Given that epigenetics is at the heart of phenotypic variation in health and disease, it seems likely that understanding and manipulating the epigenome holds enormous promise for preventing and treating common human illness. Epigenetics also offers an important window to understanding the role of the environment's interactions with the genome in causing disease, and in modulating those interactions to improve human health. ■

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