Molecular Pathology

Contents

Epigenetics

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Genomic Imprinting

Genomic imprinting is caused by alterations in chromatin in certain locations of the genome that occur in the germline of one parent, but not of the other parent. These alterations include the covalent modification of DNA, such as methylation of cytosine to form 5-methylcytosine, or the modification or substitution in chromatin of specific histone types, which can influence gene expression within a chromosomal region. The allelic expression of an imprinted gene depends upon whether it resided in the male or female of the previous generation. Imprinted expression can also vary between tissues, developmental stages, and species.

We inherit two copies of every autosomal gene, one copy from the mother and one from the father. Both copies are functional in the majority of genes. However, in a small subset, one copy is turned off in a parent-of-origin-dependent manner. These genes are called 'imprinted' because one copy of one of the genes, either from the sperm or from the egg, was epigenetically marked or imprinted. The mechanisms for imprinting are still not completely described, but it is known that epigenetic modifications occur before fertilization and that they are erased and reset during the creation of eggs and sperm.

The imprinted state persists postnatally into adulthood though hundreds of cell divisions so that only the maternal or the paternal copy of the gene is expressed. Imprinting is reversible. A *paternally* derived allele, when it is inherited by a female, must be converted in her germline so she can pass it on as a *maternally* imprinted allele to her offspring. Likewise, an imprinted maternally derived allele, when it is inherited by a male, must be converted in his germline so he can pass it on as a paternally imprinted allele to his offspring.

Control over this conversion process appears to be regulated by the imprinting centers (ICs) that are located within the imprinted regions throughout the genome; although their precise mechanism of action is not known, they may initiate the epigenetic change in chromatin, which then spreads outward along the chromosome over the imprinted region.

The phenomenon of genomic imprinting evolved in a common ancestor to marsupials and eutherian mammals over 150 million years ago with the advent of live birth. Its evolution apparently occurred because of a parental battle between the sexes to control the maternal expenditure of resources to the offspring. Paternally expressed imprinted genes tend to promote growth, while maternally expressed genes tend to suppress it. Thus, paternally expressed genes enhance the extraction of nutrients from the mother during pregnancy, whereas the maternal genome seeks to limit it. This genetic battle between the mother and the father appears to continue even after birth since mice that lack paternally expressed *Peg1* and *Peg3* have reduced maternal nurturing behavior.

Imprinted genes are susceptibility targets for numerous human syndromes, including Angelman and Prader–Willi syndromes (PWS), as well as Alzheimer's disease, autism, bipolar disorder, diabetes, obesity, and schizophrenia. It is also seen in a number of cancers: leukemia, mesothelioma, and bladder, breast, cervical, colorectal, esophageal, hepatocellular, lung, ovarian, prostate, and testicular cancers, among others.

Imprinting Defect Analysis in Prader–Willi

PWS (OMIM, 176270) is characterized by poor muscle tone (hypotonia) and feeding difficulties in the first year of life, distinct facial features (such as rounded face, almond-shaped eyes, thin upper lip, and prominent nose), underdeveloped sex organs (hypogonadism), increased appetite (hyperphagia) leading to obesity, short stature, speech and motor delays, and some degree of cognitive impairment.

The PWS is caused by the absence of expression of paternal genes from chromosome 15q11.2–q13. A number of genes in this chromosomal region are subject to genomic imprinting and are normally active only from the paternally contributed chromosome 15 (i.e., maternally imprinted by epigenetics factors). The 'paternal-only expressed 15q11–q13 region' encompasses five polypeptide coding genes (*MKRN3*, *MAGEL2*, *NECDIN*, and the bicistronic *SNURF–SNRPN*).

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Figure 1 Metaphase showing deletion of the red signal (SNRPN) probe specific for the 15q11.2 region. Courtesy of the UCLA Cytogenetics Laboratory.

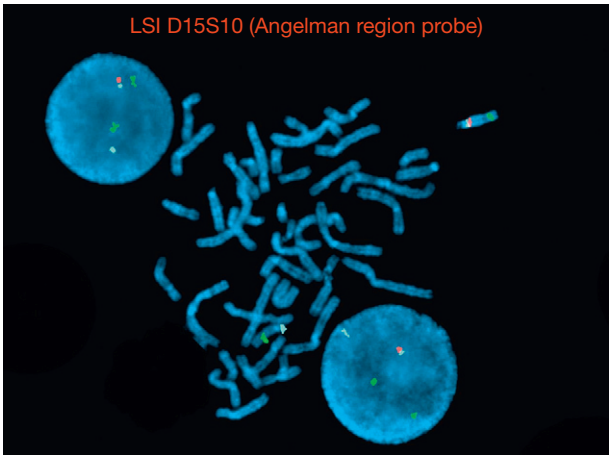

Figure 2 Metaphase showing deletion of the red signal (D15S10) Angelman region probe specific for the 15q11.2 region. Courtesy of the UCLA Cytogenetics Laboratory.

PWS is most commonly caused by a microdeletion of the paternal copy of the *SNRPN* (small nuclear ribonucleoprotein polypeptide N), *SNURF* (SNRPN upstream reading frame), and other genes located on 15q11–q13 (necdin genes along with a clusters of snoRNAs – SNORD64, SNORD107, SNORD108, two copies of SNORD109, 29 copies of SNORD116 (HBII-85), and 48 copies of SNORD115 (HBII-52) (65–75% of PW cases)). The deletion of chromosome region 15q11–q13 can be detected by florescence *in situ* hybridization (Figure 1) and chromosomal microarray analysis (CMA). About 20–30% of PW cases are due to maternal uniparental disomy (mat UPD15; two copies of the maternal chromosome 15 instead of one maternal and one paternal copy of chromosome 15). About 1–3% of PW cases have imprinting defects (IDs) due to *de novo* epigenetic mutations (80%) or a microdeletion of the IC region (7.5–100 kb) (15%). The 5′ untranslated region of the SNRPN gene has been identified as an imprinting IC and can be detected using DNA sequence analysis, CMA, and multiplex ligation-dependent probe amplification technologies. In about half of these individuals, the IC deletion is familial and the familial recurrence risk is 50%. Therefore, fathers of children with an IC deletion should have DNA methylation and dosage analysis (or sequence analysis) to determine if they carry the IC deletion.

DNA methylation studies of the *SNRPN* locus are able to detect but not distinguish all three genetic abnormalities (deletions, uniparental disomies, and imprinting deficits) that lead to PWS with an accuracy of 99%.

ID Analysis in Angelman Syndrome

Angelman syndrome (AS) (OMIM, 105830) is characterized by severe developmental delay and intellectual disability, movement and balanced disorder (ataxia), and tremulous movement of limbs, inappropriate laughter, small head size (microcephaly), and recurrent seizures.

AS is caused by the absence of expression of maternal genes on chromosome region 15q11.2–q13. The *UBE3A* (ubiquitin protein ligase E3A) and *ATP10A* (ATPase, class V, type 10A) genes in this chromosomal region are subject to genomic imprinting and are normally maternally expressed in brain and biparentally expressed in other tissues (i.e., brain-specific paternal partial imprinting by epigenetics factors).

AS is mostly caused by a microdeletion of the maternal copy of 15q11–q13 (~68%) (Figure 2). However, paternal uniparental disomy (pat UPD15; two copies of the paternal chromosome 15 instead of one maternal and one paternal copy of chromosome 15) (~7) and IDs through microdeletions (~3%) are also observed in AS cases. The characterization of the ID as either an IC deletion or an epigenetic defect is available primarily through research laboratories. About 11% of cases have mutations in the *UBE3A* gene.

ID Analysis in Beckwith–Wiedemann Syndrome

Beckwith–Wiedemann syndrome (BWS) (OMIM, 130650) is an overgrowth syndrome characterized by excessive birth weight (macrosomia), unusually large tongue (macroglossia), and embryonal tumors (e.g., Wilms tumor, hepatoblastoma, neuroblastoma, and rhabdomyosarcoma).

BWS is associated with abnormal regulation of gene transcription in an imprinted domain on chromosome 11p15.5 (also known as the BWS critical region). The BWS critical region includes two domains: IC1 regulates the expression of *IGF2* and *H19* in domain 1, and IC2 regulates the expression of *CDKN1C*, *KCNQ1OT1*, and *KCNQ1* in domain 2 (Figure 3). Genomic imprinting is a phenomenon whereby the DNA of the two alleles of a gene is differentially modified so that only one parental allele, parent-specific for each gene, is normally expressed. As shown in Figure 1, differential methylation of IC1 and IC2 is associated with the expression of specific genes on the paternal and maternal alleles in unaffected individuals.

Regulation may be disrupted by any one of numerous mechanisms: Loss of methylation at IC2 on the maternal chromosome occurs in 50% of the cases. Gain of methylation at the IC1 on the maternal chromosome occurs in 5% of the cases. *CDKN1C* mutations occur in ~40% of persons with a

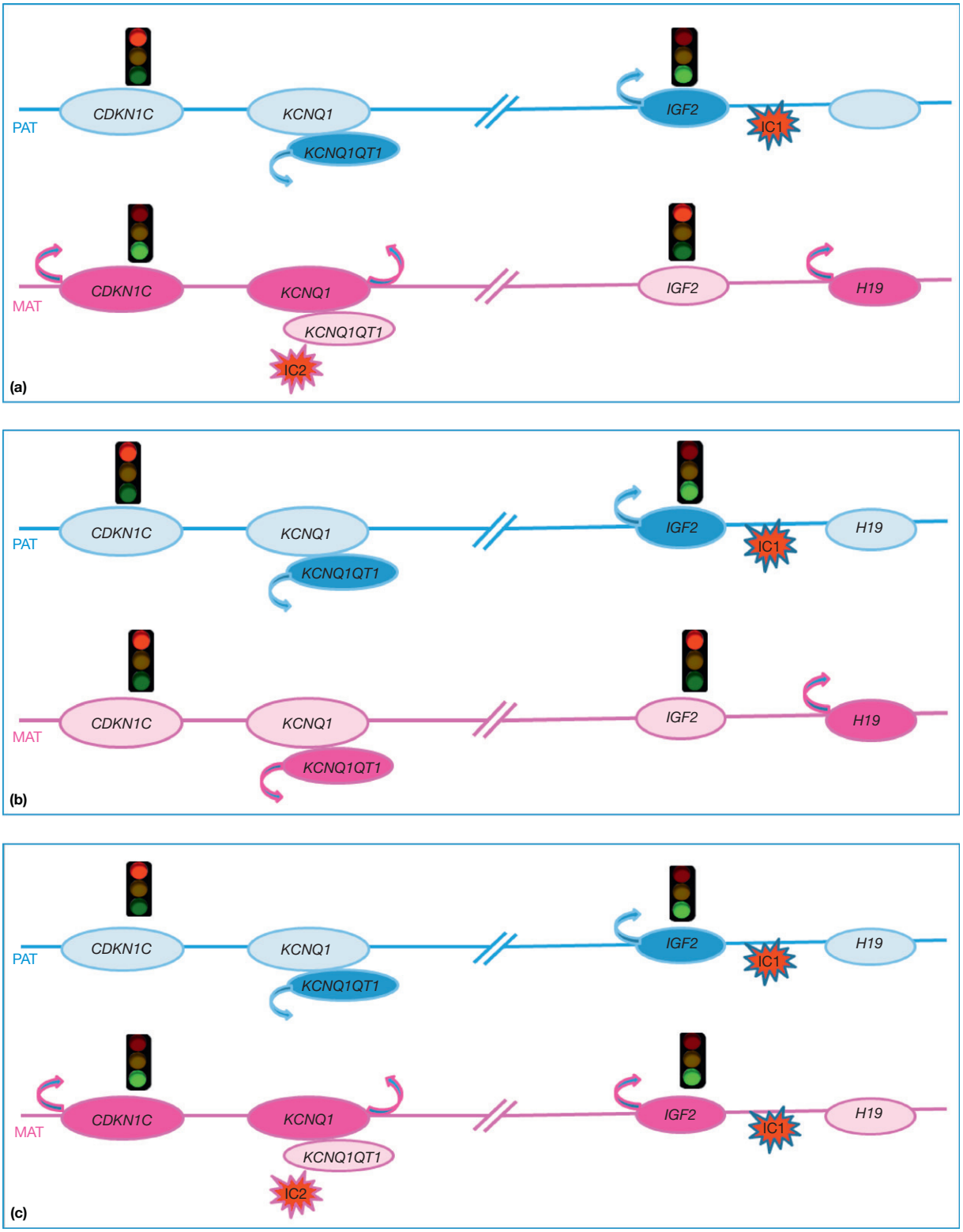

Figure 3 (a) The 11p15.5 region is functionally divided in two domains. Domain 1 has two imprinted genes: *1GF2* encoding insulin-like growth factor 2, a fetal growth factor, and *H19*, a noncoding RNA. The *H19*-associated imprinting center 1 (IC1) is usually methylated on the paternal chromosome and unmethylated on the maternal chromosome; thus, *IGF2* is expressed from the paternal allele and *H19* from the maternal allele. Domain 2 has several imprinted genes including *CDKN1C*, *KCNQ1*, and *KCNQ1OT1*. The IC2 contains the promoter for *KCNQ1OT1*, a noncoding transcript that regulates in *cis* the expression of the imprinted genes in domain 2. IC2 is usually methylated on the maternal chromosome and unmethylated on the paternal chromosome; thus, *CDKN1C* and *KCNQ1* are expressed from the maternal allele and *KCNQ1OT1* from the paternal allele. (b) This is an example of imprinting alterations leading to Beckwith–Wiedemann syndrome (BWS). IC2 loss of methylation on the maternal chromosome, found in 50% of persons with BWS, leads to reduced expression of *CDKN1C*. (c) This is another example where IC1 gain of methylation on the maternal chromosome, found in ~5% of persons with BWS, leads to the biallelic expression of *IGF2*. Adapted from Shuman, C., Beckwith, B., Smith, A., Wesberg, R., 2010. Beckwith–Wiedemann syndrome. In: Pagon, R.A., Adam, M.P., Bird, T.D., et al. (Eds.), GeneReviews. University of Washington, Seattle, WA.

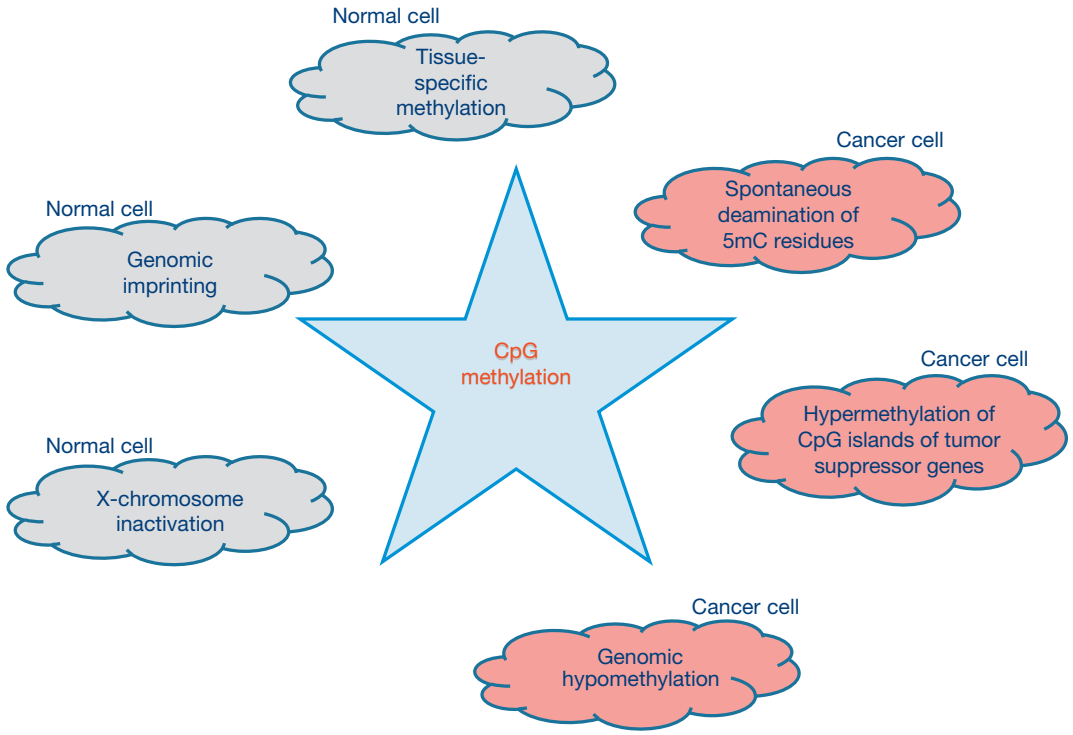

Figure 4 DNA methylation in normal and cancer cells. Adapted from Cheng, J.C., Jones, P.A., 2005. Epigenetic events in cancer. In: Knowles, M.A., Selly, P.J. (Eds.), Introduction to the Cellular and Molecular Biology of the Cancer, fourth ed. Oxford University Press, Oxford, pp. 78–88 (Chapter 5).

positive family history of BWS. Genomic alterations involving the IC1 and/or IC2 have also been seen in BWS patients. Other causes of BWS include uniparental disomy as well as other chromosomal abnormalities.

Mechanisms of Epigenetic Regulation

DNA methylation occurs differently in normal and cancer cells. In normal cells, DNA methylation is involved in tissue-specific methylation, genomic imprinting, and X-chromosome activation. Conversely, cancer cell DNA methylation involves spontaneous deamination of 5mC residues, aberrant methylation of CpG islands of tumor suppressor genes (TSGs), and hypomethylation (Figure 4).

There are three main mechanisms of epigenetic regulation in leukemias: DNA methylation, histone modification, and microRNA (miRNA) expression. The following sections will discuss the three mechanisms theoretically and then will discuss the epigenetic signatures of each of the four main types of leukemia.

DNA Methylation

The normal process of DNA methylation occurs in CpG-rich sequences, which are found in 60% of all genes. These sequences are called CpG islands and usually reside near the promoter and exogenic regions of the host genes. DNA methyltransferases (DNMTs) add methyl groups to the 5′ position of the cytosine pyrimidine rings in CGIs, blocking gene expression, possibly by blocking the attachment sites of transcription

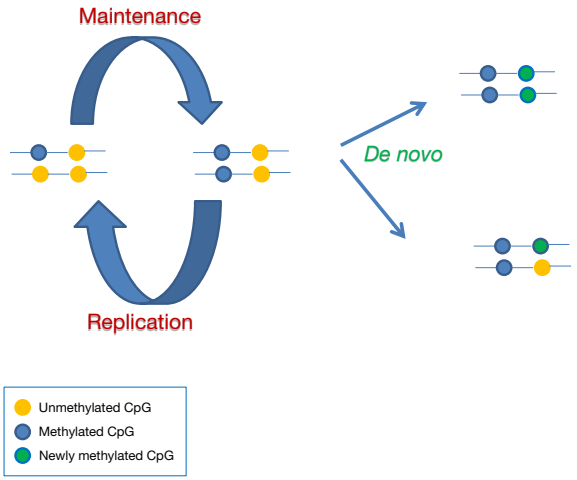

Figure 5 Maintenance of CpG methylation is required during DNA replication. Furthermore, *de novo* methylation occurs when formerly unmethylated regions are methylated. Adapted from Cheng, J.C., Jones, P.A., 2005. Epigenetic events in cancer. In: Knowles, M.A., Selly, P.J. (Eds.), Introduction to the Cellular and Molecular Biology of the Cancer, fourth ed. Oxford University Press, Oxford, pp. 78–88 (Chapter 5).

factors. Maintenance of methyltransferase activity is responsible for copying methylation patterns onto newly synthesized strands of DNA based on the methylation status of the template parent strand (Figure 5).

DNA methylation is essential for mammalian embryogenesis. Methylation patterns are established during phases of

development, showing an initial wave of genome-wide demethylation from fertilization until the eight-cell stage of blastocyst formation, which removes most of the preexisting methylation inherited from parental DNA. This is followed after implantation by a wave of *de novo* methylation.

DNA methylation also serves as an essential mechanism for permanent, heritable silencing of gene transcription in mammal development, most notably in genomic imprinting, transcriptional silencing of parasitic sequence elements, and X inactivation. An important regulatory role of DNA methylation has been seen in genomic imprinting. Differential DNA methylation is a critical signal for mammalian gene imprinting, leading to monoallelic expression of these genes. The functional differences between the paternal and the maternal genomes are attributed to the differential expression of the respective alleles of several dozen imprinted genes during development. In many clusters of imprinted genes, one allele is highly methylated and the other is not methylated or methylated at only a small percentage of CpGs in a 1–5 CpG-rich differentially methylated region (DMR). The methylation patterns of DMRs exhibit gamete-specific differences, which are usually partially retained during embryogenesis and generally appear to be primary imprinting marks. The paternal alleles of the *H19* (transforming-suppressing RNA) and *Rasgrf1* (guanine nucleotide exchange factors) genes are methylated in their 5′ upstream regions in the male germ cells during embryogenesis, whereas the other known imprinted genes such as *Igf2r* (antiapoptotic growth factor) and *Snrpn* (SMM protein involved in RNA splicing) acquire their methylation imprints from the oocyte. Deletion of such DMRs results in the loss of imprinting.

Abnormal DNA methylation in leukemia

Abnormal methylation of the DNA, both hyper- and hypomethylation, can disrupt healthy hematopoiesis, and thus, both have been implicated in leukemia. The primary oncogenic character of hypermethylation is the suppression of TSGs. For example, hypermethylation has been observed in acute myeloid leukemia (AML) patients with a highly heterogeneous pattern of methylation of the cyclin-dependent kinase inhibition gene *CDKN2B* (p15) and is associated with leukemogenesis and progression of AML.

Hypomethylation of proto-oncogenes can also lead to leukemia. For example, hypomethylation of the T-cell leukemia/lymphoma 1 gene (*TCL-1*) has been observed in chronic lymphocytic leukemia (CLL). Table 1 shows other cases of abnormal methylation. Discussion of each gene follows in the sections on the respective leukemias in which the abnormal methylation is observed.

Histone Modification in Leukemia

Histone acetyltransferases (HATs), histone deacetylases (HDACs), and histone methyltransferases (HMTs) add chemical groups to the histone octamers around which the DNA is wound into chromatin. Chemical modification of histone tails changes the DNA between euchromatin and heterochromatin. Acetylation is associated with euchromatin and transcriptional activation and deacetylation with heterochromatin and transcriptional repression. Methylation is not as well directly correlated with euchromatin or heterochromatin. Abnormal expression of HATs, HDACs, and HMTs affects DNA association with the histone proteins producing changes in gene expression that could lead to cancer and specifically, in hematopoiesis, to leukemia.

As an example, Minucci et al. and by Lin et al. observed constitutive recruitment of HDACs to retinoic acid (RA) target genes pursuant to the irreversible binding of RA receptor (RAR) fusion proteins to the *NCoR/SMRT* protein. This led to acute promyelocytic leukemia (APL), a subclass of AML. This repression of RA target genes blocks hematopoietic differentiation, resulting in leukemogenesis. Improper functioning of HATs has also been associated with leukemia, as demonstrated by Mullighan et al. in which 18% of relapsed AML cases displayed

Table 1 Abnormal DNA methylation in leukemias

Gene	*Locus*	*Function*	*Associated leukemia*	*Methylation prevalence (%)*	*References*
ABL1	9q34	Kinase	ALL, CML	33, 23–42	Shteper et al. (2001) and Issa et al. (1999)
APC2	19p13	Wnt signaling	CLL	77	Rahmatpanah et al. (2009)
ARHGAP20	11q23	Small G protein	ALL	65/33	Dunwell et al.(2010)
BIM	2q13	Apoptosis inductor	CML	44	San Jose-Eneriz et al. (2009)
BRCA1	17q21	DNA repair	AML	29/75	Scardocci et al.(2006)
CDC14B	9q22	Cell cycle	ALL	44/33	Dunwell et al.(2010)
CDKN2B	9p21	Cell cycle control (G1–S)	ALL, AML, CLL	23–50, 30–70, 50	Garcia-Manero et al. (2009) and Melki and Clark (2002)
CDKN2A	9p21	Cell cycle control (G1–S)	ALL, AML, CLL	7/22, 25, 20	Matsushita et al. (2004) and Melki and Clark (2002)
DAPK1	9q34	Cell death	AML, CLL	61, 100	Ekmekci et al. (2004) and Raval et al. (2005)
DMRT2	9p24	Sex determination	CLL	47	Rahmatpanah et al. (2009)
p73	1p36	Cell cycle control (G1–S)	AML	10–37	Ekmekci et al.(2004)
RASSF10	11p15	Ras signaling	ALL	16/88	Hesson et al.(2009)
TP53I11	11p11	P53 signaling	ALL	15/25	Dunwell et al.(2010)
UBE2C	20q13	Cell cycle control (G2–M)	ALL	23/17	Dunwell et al.(2010)
WIT-1	11p13	Transcription factor	AML	37	Plass et al. (1999)

Source: Florean, C., Schnekenburger, M., Grandjenette, C., Dicato, M., Diederich. M., 2011. Epigenomics of leukemia: from mechanisms to therapeutic applications. Epigenomics 3 (5), 581–609.

incapacitating mutations in the HAT domain of an acetylating protein, CREB-binding protein (CBP). Improper methylation of histones has also been implicated in leukemogenesis. Hess showed that some translocations generate the HMT fusion protein, *MLL*–AF10, that aberrantly recruits the HMT hDOT1L (human disruptor of telomeric silencing-1L) to methylate the H3K79 histone of the HOX genes. The overexpressed HOX genes may trigger leukemogenesis.

miRNA in Leukemia

miRNAs have also been implicated in leukemias. Expression of miRNAs can be leukogenic in two ways: first, suppression of a given miRNA could lead to the overexpression of target genes (possibly an oncogene) and, second, overexpression of a given miRNA could prevent the suppression of TSG transcripts, blocking the production of the anticancer protein.

One example of miRNA implicated in leukemia is reported by Zhao et al. in CLL. The most common deletion characteristic of CLL, del13(q14), downregulates the production of miR15a/16-1. Without miR15a/16-1, the antiapoptotic factor Bcl-2 is overexpressed, and leukemogenesis commences.

Epigenetic Signatures in AML

AML displays hypermethylation in several TSGs, including *HIC1*, homeobox gene *HOXA4*, transcription factor *WIT-1*, and most notably, *CDKN2B* (p15). Hypermethylation of these genes silences them as effectively as if they had been cytogenetically deleted. Loss of expression of these TSGs forfeits control over their respective target genes. *HIC1* is a transcriptional repressor, but is not expressed when hypermethylated, leading to overexpression of its target genes, including as *SIRT1*, *CCND1*, and *CDKN1C*, as well as reduced regulation of the Wnt signaling pathway, in which *HIC1* exerts some control. *HOXA4* governs segmental differentiation as a homeobox gene, but is not expressed for its task when hypermethylated. In addition to its role in the development of the urogenital system, *WIT-1* also binds to the p15 and acts as a TSG. Thus, hypermethylation of *WIT-1* deprives the cell of the function of that particular TSG. *CDKN2B* (p15) suppresses the activity of CD kinases, regulating cell growth at the G1 stage of the cell cycle: the hypermethylation of *CDKN2B* removes that control of the cell cycle.

Two hypotheses have been proposed to explain the mechanism of hypermethylation in AML. The first suggests the overall increased expression of DNMTs, which are the proteins that add the methyl groups to the 5′ carbon of the cytosine pyrimidine ring to block transcription. The second hypothesis from Di Croce et al. is more specific. They suggest that the characteristic chimera protein of AML, *PML/RARA*, resulting from t(15;17), uncontrollably recruits *DNMT1* and *DNMT3* to the promoters of TSGs (specifically *RARb2*), eliminating expression of those genes, as effectively as if those genes had been deleted.

Hypomethylation in AML, on the other hand, overexpresses proto-oncogenes. Some proto-oncogenes hypomethylated in AML include the tyrosine kinase c-fms, the myeloperoxidase (MPO), and the GTPase *H-RAS*. c-fms codes for a membrane protein that receives the M-CSF protein, which regulates the production, differentiation, and function of macrophages. Overexpression of c-fms by hypomethylation results in excessive response to the presence of M-CSF. MPO is a lysosomal protein that neutrophil granulocytes use to destroy pathogens. Hypomethylation of MPO overexpresses that gene and the cytotoxic chemicals it produces, which can be catastrophic for the cell. *H-RAS* is the first of signaling proteins in the cell growth pathway. Hypermethylation forfeits the restraint on cell growth and multiplication that characterizes the function of *H-RAS*.

The mechanism of hypomethylation remains unresolved, but several theories have been proposed. Shah and Licht proposed the most thorough hypothesis that states that hypomethylation may be the result of mutations in *DNMT3A*. Those mutations, found in 22.1% of AML patients, may inactivate its methyltransferase activity, but this effect is debated in the literature. There is no clear answer to how *DNMT3A* mutations affect DNA methylation levels.

There are three types of epigenetic signatures for histone modification in AML, each having to do with aberrant recruitment or expression of HDACs, HATs, or HMTs. In APL, HDACs are overrecruited to RA target genes as a result of the interaction between *PML/RARA* chimera protein, the product of t(15;17), and the correpressive complex *NCoR/SMRT*, with which *PML/RARA* constitutively binds. RARs normally suppress transcription, but when RAR fuses with PML as a result of t(15;17), RAR cannot disassociate from *NCoR/SMRT*, and deacetylation cannot be stopped. Sustained deacetylation of RA genes blocks hematopoietic differentiation. Non-APL AML cases express t(8;21), *RUNX1/RUNX1T1*, which also excessively recruits HDACs by a similar mechanism to that of t(15;17), or *PML/RARA*. Abnormal HAT functioning is also correlated with cytogenetic rearrangements. CPB protein has a HAT region, which is mutated in 18% of relapsed AML cases, suggesting that functioning HATs are necessary for proper response to therapy. Also, the *MOZ–CBP* fusion protein, resulting from t(18;16), mistargets genes and may acetylate – and hence activate – the wrong genes. Finally, HMT overrecruitment in AML has been shown to result from *MLL* fusions, leading to the overexpression of HOX genes, which *MLL* regulates.

The roles of miRNAs or miRs are relatively unexplored, leaving a gap in the understanding of epigenetic control in AML. Patterns of miR expression have been correlated with subtypes of leukemias, but no causal relation or mechanism has been demonstrated between miRs and leukemogenesis. In AML, various patterns of miR expression have helped distinguish between different types of AML, such as those bearing t(15;17), t(8;21), Inv(16), NPM1, CEBPA, FLT3-ITD, or *MLL* rearrangements.

Recently developed treatments for AML use epigenetics in conjunction with conventional chemotherapy. Some epigenetic therapies for AML include the hypomethylating agent, 5-azacitidine, marketed as Vidaza, which has been shown in a phase II study to improve myelodysplastic syndrome and AML patients. HDAC inhibitors have also proven effective in the treatment of AML, such as treatments with valproic acid that induced differentiation and entinostat in combination with 5-azacitidine that improved cytotoxicity.

Epigenetic Signatures of CML

Chronic myelogenous leukemia (CML) displays hypermethylation in a set of TSGs as it progresses from chronic phase (CP) to blast crisis (BC). These genes include *HIC1*, *CALC1* involved in calcium metabolism, the kinase *ABL1*, homeobox gene *HOXA4*, and apoptosis inducer *BIM*. The functions of *HIC1* and *HOXA4* were already described in the section on the epigenetics of AML. *CALC1* produces a protein that keeps calcium blood levels low, in one way, by inhibiting the activity of osteoclasts. *ABL1*'s protein product regulates cell growth, DNA damage response, and apoptosis. Hypermethylation silences the transcription of *ABL1* as effectively as if it had been cytogenetically deleted, removing *ABL1*'s control over its functions. *BIM* induces apoptosis. Thus, the problems of hypermethylation are obvious, resulting in an old cell that continues to replicate but will not die. The mechanism of hypermethylation in CML is less well studied than that in AML, but it has been observed that the levels of DNMT expression proportionately reflect the progression of CML, showing a spike in expression when the leukemia progresses from CP to BC.

Abnormal hypomethylation leads to overexpression of genes in CML. *LINE1* retrotransposons and the cancer antigen *HAGE* display markedly higher expression in BC of CML compared with CP, as a result of hypomethylation. *CD7*, which codes for transmembrane proteins in developing and mature T-cell lymphocytes, is also overexpressed when hypomethylated and has been used in early prognosis of CML.

Literature on histone modification in the epigenetics of CML is sparse, and research has not gleaned many applications to understanding leukemogenesis or to medical therapy in this area.

miRs have also shown patterns of expression reflecting the progression of CML into BC. Specifically, miRs controlling *MYC* and *BCR–ABL* transcripts are downregulated as CML proceeds to BC. Also, in general, CML exhibits downregulation of miRs that control *ABL1* transcripts, thereby upregulating *ABL1* expression. Some patterns of miR expression have even been used to predict response to imatinib therapy in CML.

CML treatments have also begun to use epigenetic therapy. Hypomethylating agents, including 5-azacitidine (Vidaza®) and 5-aza-2′-deoxycytidine (also called decitabine and marketed as Dacogen®), have been reported to increase response to imatinib therapy in CML cases with hypermethylated *BIM*. Schnekenburger et al. have shown that decitabine also induces autophagy and senescence in CML cells.

Epigenetic Signatures of ALL

Some TSGs are also hypermethylated in acute lymphoblastic leukemia (ALL), including *CDKN1A* (p21), calcium metabolism gene *CALC1*, and the kinase *ABL1*. The functions of *CALC1* and *ABL1* have already been described earlier. *CDKN1A* inhibits the cell cycle at the G1 boundary in the case of DNA damage. Hypermethylation silences these three genes, blocking their ability to decelerate or halt the cell cycle. The hypermethylation state of *ABL1* is particularly noteworthy in ALL, because it identifies a particular subtype of Philadelphia chromosome-positive (Ph+) ALL. Homeobox gene *HOX11* is the only gene displaying a hypomethylated pattern in ALL. *HOX11* is associated with the development of the spleen, but overexpression has been associated with T-cell ALL.

Aberrant HDAC and HMT activity has also been observed in ALL. Several groups have associated overexpression of HDAC transcripts with different subtypes of ALL. While no direct HDAC activity has been observed, it makes sense that increased HDAC activity follows upon the overexpression of HDAC transcripts. Aberrant HMT expression results from translocations involving *MLL*, which has HMT ability. The mutated *MLL* product protein no longer properly regulates its usual target *HOXA* genes, leading to abnormal cell differentiation and proliferation.

miR expression in ALL assists in both diagnosis and prognosis. Patterns of miR expression have been used to distinguish between AML and ALL with 97% accuracy, as well as to predict resistance to vincristine and daunorubicin in childhood ALL.

Epigenetic therapy for ALL, using the HDAC inhibitor entinostat together with 5-azacitidine, has entered clinical trials with greater cytotoxicity in transformed cells.

Epigenetic Signatures of CLL

The most useful prognostic tool in CLL is the mutational status of the *IgVH* gene, which codes for immune antigens. High mutational status in *IgVH* indicates a fair prognosis, while low mutational status indicates a poor prognosis. Several genes display a hypermethylation pattern correlated with a highly mutated *IgVH* and hence a fair prognosis in CLL. Thus, the methylation status of these genes can be used as a prognostic proxy in CLL. Hypermethylated – that is, silenced – genes correlated with high *IgVH* mutational status include *ZAP-70*, which functions in T-cell signaling; *TWIST2* (*Dermo-1*), which regulates osteogenesis; *BTG4*, a TSG that induces G1 cell cycle arrest; and *CD38*, which codes for glycoproteins on immune cells. There is one gene, though, whose hypermethylation status correlates with a low *IgVH* mutational status and hence a bad prognosis: *HOXA4*. Hypomethylation, on the other hand, has been observed activating the oncogene *TCL-1*, the GTPase *H-RAS*, *DNMT* genes, and LINE1 retrotransposons.

The only epigenetic histone modification observed in CLL is the methylation of H3, silencing the RELB gene, which codes for a transcription factor ubiquitous to many cell types, processes, and functions. This methylated state of the histone correlates with therapy resistance in male patients.

miR epigenetic signatures have been correlated with the three most prominent cytogenetic signatures of CLL: del13 (q14), ZAP-70 variations, and mutational status in IgVH. This suggests that miRs are promising tools for prognosis in CLL.

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Further Reading

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