Cancer epigenetics

In addition to having genetic causes, cancer can also be considered an epigenetic disease.

Regulation by genetics involves a change in the DNA sequence, whereas epigenetic regulation involves alteration in chromatin structure and methylation of the promoter region.
The inheritance of information on the basis of gene expression levels is known as epigenetics, as opposed to genetics, which refers to information inherited on the basis of gene sequence.

**Epigenetics** - qualitative changes in genomic content.

**How information might be stably maintained through multiple rounds of cell division?**

The heritability of newly acquired traits is a hallmark of clonal expansion in tumours.
The three main types of epigenetic information

**Cytosine DNA methylation** is a covalent modification of DNA, in which a methyl group is transferred from S-adenosylmethionine to the C-5 position of cytosine by a family of cytosine (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.

2/9/2006

**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.

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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.

Enzymatic methylation of the C–5 position of cytosine residues can effect epigenetic inheritance by altering the expression of genes and by transmission of DNA methylation patterns through cell division.

Thus, in addition to its well-known role in deamination mutational hotspots in human DNA, DNA methylation may contribute to gene inactivation in cancer.
Gene silencing

DNA methylation is a powerful mechanism for the suppression of gene activity.

There is reciprocal relationship between the density of methylated cytosine residues and the transcriptional activity of a gene

The methyl groups do not affect base pairing but can influence protein–DNA interactions by protruding into the major groove.
The strong effect of 5–methylcytosine (5mC) in mammalian promoter regions suggests that DNA methylation inhibits transcription by interfering with transcription initiation.

DNA methylation reduces the binding affinity of sequence-specific transcription factors.

Methylation–dependent, sequence–specific DNA–binding proteins, such as MDBP may act as transcriptional repressors.

**Paradox:**
The range of transcription factors that can be influenced directly by DNA methylation is likely to be limited to the subset containing the CpG sequence in their binding site, yet DNA methylation seems to affect a wide diversity of promoter sequences.
Explanation to the ‘paradox’:

Transcriptional repression by DNA methylation can be mediated through a sequence–independent process involving changes in chromatin structure and histone acetylation levels.

1. The transcriptional repressor MeCP2 binds methylated DNA in a sequence–independent fashion.

2. Transcriptional repression by MeCP2 is non–linearly dependent upon 5mC density and involves recruitment of a complex containing a transcriptional co–repressor and a histone deacetylase.

3. Deacetylation of histones is associated with reduced levels of transcription, perhaps by allowing tighter nucleosomal packing.

Mechanism of transcriptional repression by DNA methylation.

The structure of transcriptionally active chromatin is shown schematically at the top. Nucleosomes are shown as blue cylinders representing the core histone complexes with DNA indicated by the black line wrapped around the cylinders. Yellow ovals (Ac) denote acetylation of histones. DNA methylation (Me) is indicated by red circles.
Methylation changes - a third pathway to loss of function

Knudsen’s hypothesis that two hits are required for the full inactivation of a tumour–suppressor gene has been shown to be fundamentally correct in almost all cases of human cancer.

The focus has until now been on two pathways by which suppressors become disabled: (1) intragenic mutations and (2) loss of chromosomal material (loss of heterozygosity (LOH) or homozygous deletion).

However, the fact that methylation of CpG islands located in the promoters of genes can cause transcriptional silencing, coupled with the observation that DNA methylation patterns are perturbed in cancer cells, has lead to the suggestion that abnormal methylation of the promoters of tumour–suppressor genes might be implicated in carcinogenesis.

The frequency with which promoter methylation contributes to gene inactivation ranges from about 9% of RB1 in retinoblastoma to 33% of VHL in Von Hippel Lindau (VHL) disease to 84% of MLH1 in microsatellite–unstable colorectal tumours.

Promoter methylation therefore represents a significant contributor to human carcinogenesis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tumour type</th>
<th>Mechanisms of inactivation</th>
<th>Documentation of double hits</th>
<th>References</th>
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<td></td>
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<td>yes</td>
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<td>yes</td>
</tr>
<tr>
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<tr>
<td>APC</td>
<td>colorectal cancer</td>
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</tr>
</tbody>
</table>
To date, **more than 600 genes**, including tumor suppressor genes, oncogenes, and cancer-associated viral genes, have been reported to be regulated by epigenetic mechanisms.

For example, these genes include APC, ER, RAR, p15, p16, p73, DAPK1, E-cathedrin, GSTP1, LKB1, MGMT, TIMP3, and VHL.

Four lines of evidence suggest a causal role for DNA methylation at the preneoplastic stages of cancer development:

1. Reduction of methylation suppresses the formation of intestinal polyps in ApcMin/+ mice.
2. Abnormal biallelic methylation of the H19 gene promoter is seen in the pre-neoplastic kidney parenchyma of Wilms tumour patients. This is accompanied by extinction of H19 expression and upregulation of IGF2, and is an epigenetic change which cannot reflect changes in the tumours as the tissue is non-neoplastic.
3. Methylation of the promoter of wild-type VHL has been documented in familial cases of VHL syndrome as well as in the promoter of RB1 in unilateral retinoblastoma.
4. Role of methylation in sporadic cases of mismatch repair-deficient colorectal carcinomas. These carcinomas with microsatellite instability (MSI) have hypermethylation of the promoter CpG island of the mismatch repair gene MLH1. Treatment of cell lines derived from such tumours with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-azad-C) leads to demethylation of the MLH1 promoter, reaccumulation of the MLH1 protein and restoration of mismatch-repair activity. Thus - CpG island hypermethylation is a primary event in the inactivation of MLH1.
Clinical prospects

Reversion of epigenetic changes to restore gene function?

- Treatment with DNA methylation inhibitors can restore the activities of dormant genes such as CDKN2A and decrease the growth rate of cancer cells in a heritable fashion.
- DNA repair capacity can be restored by activation of MLH1 by the use of methylation inhibitors.
- Min mice - inhibition of DNA methylation can suppress tumour initiation

DNA methylation may also have applications in diagnostics.

- DNA methylation is the only epigenetic mark preserved in genomic DNA isolated from tumours or biopsies.
- To the extent that DNA methylation patterns reflect the expression status of genes, DNA methylation could be used as a surrogate for RNA- or protein-based expression analyses of tumours, both of which are difficult in paraffin-embedded archival tumour samples.

Mechanisms and important players

DNMTs

MeCP2 - can repress transcription of methylated genes by recruitment of a corepressor complex that contains histone deacetylases.

The MeCP2 gene turns out to be clinically important, as mutations within it are the primary cause of Rett Syndrome, a severe inherited neurological disorder that affects girls.

MBD1-4

MBD2 a repressor of methylated genes, is required for tumorigenesis in a mouse model of human familial colorectal cancer. Although mice lacking MBD2 are healthy, tumorigenesis is dramatically reduced. Anti-MBD2 drugs - novel potential anti-cancer agents?

Like MeCP2 and MBD2, MBD4 also has a methyl-CpG binding domain. Possibly - DNA repair protein? Its primary role is to initiate repair of deaminated 5-methylcytosine residues, which are a major source mutations that cause human genetic disease. MBD4-deficient mice have an increased number of mutations at methylated sites in the genome, and are more susceptible to cancer.
MECHANISMS OF TARGETING DNMT1, A DNA METHYLTRANSFERASE, TO DNA REPLICATION FOCI

DNMT1 is a large DNA cytosine methyltransferase of 1618 amino acids. Its catalytic domain resides its C-terminus, while its N-terminal domain serves a regulatory function. In cells, DNMT1 localizes to DNA replication foci. Inactivation of DNMT1 by siRNA in normal or cancer cells leads to loss of DNA methylation in both cases and re-activation of silenced tumor suppressor genes in cancer cells. Therefore, DNMT1 is required for maintaining DNA methylation in normal and cancer cells. Three regions in the N-terminus of DNMT1, replication target regions (RTRs), are involved in recruitment of DNMT1 to replication foci.

A DNA replication fork

http://mayoresearch.mayo.edu/mayoresearch/zhang_lab/targeting_Dnmt1.cfm
**DNMT3A** and **DNMT3B** are required for new DNA methylation and normal development in mice. **DNMT3B** is mutated in human ICF syndrome, a rare genetic disease associated with decreased DNA methylation. **DNMT3A** and **DNMT3B** are expressed in early embryos and able to methylate various native and synthetic DNA substrates.

**Knockout studies** - no mice were born from the group missing both genes. By the eighth day of development, embryos were small and grossly abnormal (see figure), and none survived past midgestation. No viable mice were recovered from the group missing only the **DNMT3B** gene, although embryonic abnormalities were not evident until later stages. Mice with only the **DNMT3A** deletion developed to term and appeared normal at birth, but they failed to grow and died at about four weeks. Inactivation of both genes also blocked *de novo* DNA methylation in early embryos. Yet methylation did occur in embryos missing only the **DNMT3A** or **DNMT3B** genes. Possibly two proteins methylate different sequences in the genome. **DNMT3B** specifically methylated sequences in the centromeric region – this might have an important role in maintaining chromosome stability.

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**MBDs target corepressor complexes to methylated DNA.**

On the left side are different situations that the MBDs may recognize. Empty circles represent unmethylated CpGs, whereas full circles correspond to their methylated status. (A) corresponds to hypomethylated DNA with occasional hemi-methylated CpGs. (B) corresponds to fully methylated sequence with a low density of CpGs. (C) and (D) are two sequences with a high number of CpGs but with different organizations that may be recognized by different MBDs. (E) includes the structure of the chromatin.
Transcription in eukaryotic cells is influenced by the manner in which DNA is packaged.

In resting cells, DNA is tightly compacted to prevent accessibility of transcription factors. DNA is packaged into chromatin, a highly organized and dynamic protein–DNA complex.

The fundamental subunit of chromatin, the nucleosome, is composed of an octamer of four core histones, i.e. an H3/H4 tetramer and two H2A/H2B dimers, surrounded by 146bp of DNA.
**Histones**

- 5 types:
  - H2A, H2B (slightly lys rich),
  - H3,
  - H4 (arg rich)
  - H1 (lys rich). All relatively small proteins.

- Per 200 bp of DNA: 2 molecules each of H2A, H2B, H3, H4 and one molecule of H1.

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**Histone Acetylation**

Acetylation of the lysine residues at the N terminus of histone proteins removes positive charges, thereby reducing the affinity between histones and DNA. This makes RNA polymerase and transcription factors easier to access the promoter region. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

[Diagram of histone acetylation and deacetylation]
Transcription process and its regulation by histone modification

A) Schematic representation of a nucleosome.
B) Transcriptional repression and activation in chromatin. Both histone acetyltransferase (HAT; activation) and HDAC (repression) require several cofactors (for DNA binding, for recruitment of the complex, for remodelling of the DNA helix to reduce the accessibility of transcription factors) for their activity.

The architecture of chromatin is strongly influenced by post-translational modifications of the histones

Compared with methylation and phosphorylation, the acetylation of core histones is probably the best understood type of modification.

Histone acetylation occurs at the ε amino groups of evolutionarily conserved lysine residues located at the N-termini.

All core histones are acetylated in vivo; modifications of histones H3 and H4 are, however, much more extensively characterized than those of H2A and H2B.

Important positions for acetylation are Lys9 and Lys14 on histone H3, and Lys5, Lys8, Lys12 and Lys16 on histone H4.

Steady-state levels of acetylation of the core histones result from the balance between the opposing activities of histone acetyltransferases and histone deacetylases (HDACs).
The histone acetylation switch

Targeted HAT and HDAC activities negotiate the acetylation status of chromatin. Acetylation establishes a structure that permits ATP-dependent chromatin remodeling factors to open promoters. Deacetylation, frequently followed by histone methylation, may form a solid base for highly repressive structures, such as heterochromatin. Acetylated histone tails are shown as yellow circles. Methylations are indicated as gray rectangles. 

HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; HP1, heterochromatin protein 1

An example of transcriptional activation by histone acetylation. p300/CBP can interact with a variety of transcriptional regulators such as pCAF (a histone acetyltransferase) and TBP (which recognizes the promoter). During transcription, they are all assembled at the promoter region. Histone acetylation by p300/CBP. pCAF and TAF\textsubscript{II}250 facilitates the transcription.
An example of transcriptional repression by histone deacetylation. The repressor Mad/Max dimer interacts with SIN3 (or N-CoR, SMRT, etc.) which recruits histone deacetylase (HDs) to repress transcription.

2/9/2006

Histone acetylation and cancer

The fact that acetylation is a key component in the regulation of gene expression has stimulated the study of HDACs in relation to the aberrant gene expression often observed in cancer.

Although no direct alteration in the expression of HDACs has yet been demonstrated in human oncogenesis, it is now known that HDACs associate with a number of well characterized cellular oncogenes and tumour-suppressor genes [e.g. Mad and retinoblastoma protein (Rb)], leading to an aberrant recruitment of HDAC activity, which in turn results in changes in gene expression.

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In acute promyelocytic leukaemia:

The oncoprotein produced by the fusion of the \textit{PML} (promyelocytic leukaemia) gene and the \textit{retinoic acid receptor a} gene appears to suppress the transcription of specific genes through the recruitment of HDACs.

Thus the cancer cell is unable to undergo differentiation, leading to excessive proliferation.

Similar phenomena:
retinoic acid receptor a–PLZF (promyelocytic leukaemia zinc finger protein) fusion, AML1 (acute myelocytic leukaemia protein 1)–ETO fusion, and also in the Myc/Mad/Max signalling pathway involved in solid malignancies.

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It is clear that HDAC enzymes seldom operate alone.
Many proteins, with various functions such as recruitment, co-repression or chromatin remodelling, are involved in forming a complex that results in the repressor complex.

Link between DNA and histone changes:
The most important signal involved in the initiation process of repression is situated in the DNA itself.
Methyl groups bound to the cytosine residues situated 5´ to guanosines in DNA, in so-called CpG islands, are directly responsible for the recruitment of the HDAC complex via proteins such as methylated-CpG-binding proteins and methyl-CpG-binding-domain- containing proteins, or via the enzymes that methylate the CpG islands, the DNA methyltransferases.

The methyl groups provide the basis for epigenetic gene silencing, such as imprinting and X chromosome inactivation, since a high proportion of methylated CpG will result in a loss of expression of the gene encoded by this stretch of DNA. Although it seems that HDAC could be solely responsible for the repression of gene transcription via recruitment to methylated CpG, this is not the case. When HDAC activity is inhibited, the transcription of the gene under study is not always (completely) restored.
There are two protein families with HDAC activity: the recently discovered SIR2 family of NAD+-dependent HDACs and the classical HDAC family.

Members of the classical HDAC family fall into two different phylogenetic classes - class I and class II

The class I HDACs (HDAC1, 2, 3 and 8) are most closely related to the yeast *(Saccharomyces cerevisiae)* transcriptional regulator RPD3.

Class II HDACs (HDAC4, 5, 6, 7, 9 and 10) share domains with similarity to HDA1, another deacetylase found in yeast .

Recently a new member of the HDAC family has been identified, HDAC11.

Evolutionary relationship between the HDACs

The class I HDACs are related to yeast *(Saccharomyces cerevisiae)* RPD3, and the class II HDACs are related to the yeast HDA1 enzyme. RPD3 is most related to HDAC1 and HDAC2; HDA1 is most closely related to HDAC6.
To exert their function, HDACs need to be in the nucleus, where their predominant substrate is found.

The nuclear localization of HDACs occurs via a nuclear localization signal (NLS) or via co-localization together with other proteins/HDACs.

Most HDACs contain a NLS, but some can be cytosolic as well; this depends on other regulatory domains.

**Class I HDACs are found almost exclusively in the nucleus**

The localization of HDAC1 and HDAC2 is exclusively nuclear, due to the lack of a nuclear export signal (NES).

HDAC3, however, has both a nuclear import signal and a NES, suggesting that HDAC3 can also localize to the cytoplasm.

Interestingly, HDAC3 is nearly always localized in the nucleus in studies described so far, which might be explained in part by the recruitment of the HDAC3 complex by HDACs 4, 5 and 7 when they are bound to the DNA via co-repressors.

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14-3-3 protein (red) can retain HDAC4/5 or HDAC7 in the cytoplasm when they are phosphorylated. CaMK is involved in nuclear export via the calcium/calmodulin signalling pathway. Subcellular localization of (A) class I HDACs and (B) class II HDACs is shown. (C) Shuttling of HDAC4, HDAC5 and HDACs7 during muscle differentiation.
HDACs appear to be key enzymes in the regulation of gene expression.

HDAC function seems to be regulated by its intrinsic features, abundance, cellular compartmentalization and association with cofactors.

Each cell type requires a specific gene expression pattern, thus prescribing a certain requirement of HDAC expression.

Surprisingly, cellular transformation and oncogenesis did not result in major changes in HDAC expression.

In the tumour tissues examined, at least one HDAC proved to be present.

The intra-tissue variation in HDAC expression might underlie the great variation in responses noted so far in in vitro studies and in clinical trials with HDACi.

**General HDAC expression per 100000 tags by tissue type**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDAC 1</td>
<td>HDAC 2</td>
</tr>
<tr>
<td>All normal</td>
<td>1.4</td>
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</tr>
<tr>
<td>Brain</td>
<td>0.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Breast</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colon</td>
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<td>5.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.8</td>
<td>11.9</td>
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<tr>
<td>Ovary</td>
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<td>3.1</td>
</tr>
<tr>
<td>Pancreas</td>
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<td>1.5</td>
</tr>
<tr>
<td>Prostate</td>
<td>1.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Heart*</td>
<td>–</td>
<td>2.3</td>
</tr>
</tbody>
</table>

All tumour

| All tumour      | 1.75    | 4.1     | 1.95    | 0.55  | 0.19   | 3.4    | 5.5    | 2.15  | 1.0    | 1.3    |
| Brain           | 1.9     | 3.8     | 2.5     | 0.9   | 6.1    | 6.1    | 7.2    | 1.5   | 1.7    | 3.1    |
| Breast          | 0.2     | 3.0     | 3.1     | 0.1   | –      | –      | –      | 8.9   | 3.2    | 4.3    |
| Colon           | 1.8     | 2.3     | 1.5     | 0.6   | 1.6    | 3.7    | 2.0    | 1.5   | 2.6    | 2.5    |
| Neuroblastoma   | 0.5     | 9.1     | 2.0     | 0.5   | –      | 8.5    | 1.5    | 1.0   | 1.5    | ND     |
| Ovary           | 2.6     | 6.1     | 2.6     | 1.0   | 3.1    | –      | 4.2    | 2.9   | 1.0    | 2.6    |
| Pancreas        | 2.3     | 2.3     | 3.0     | 1.5   | 2.9    | 7.95   | 3.0    | 1.5   | 0.8    | 2.8    |
| Prostate        | 2.1     | 3.2     | 1.9     | 0.2   | 2.4    | 3.8    | 2.6    | 1.6   | –      | 2.95   |
| Heart*          | ND      | ND      | ND      | ND    | ND     | ND     | ND     | ND    | ND     | ND     |

ND, no data available.

HDAC expression in various normal and cancerous cells is shown. The values are calculated averages. All normal and ‘all tumour’ represent the overall averages of all normal and tumour tissues respectively found in the Human Transcriptome Map (http://www.amc.uva.nl).
A wide variety of processes are associated with the inhibition of HDACs, such as apoptosis, necrosis, differentiation, inhibition of proliferation and cytostasis.

In addition, drug resistance can be overcome, and the restoration of expression of silenced genes is known to occur.

Although few studies have been performed with normal healthy cells, animal experiments and clinical trials have reported few or no side effects of the tested HDACi within the therapeutic range.

The efficiency of HDACi is greatly dependent on their stability in vivo. Most HDACi are not very stable due to their innate structure, and can also be readily degraded by first-pass liver metabolism.

Currently, many efforts are being made to expand our knowledge of the HDACs and to develop potent and stable HDACi.

In the future, this might give rise to the tailored use of HDAC-specific HDACi in order to dissect the complex functions of HDACs in a cell-type-specific manner.

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RESPONSIVE GENES

By using HDACi in in vitro model systems, effects on the expression of many genes have been documented.

The general effect often seen in cell lines is cytotoxicity, differentiation, inhibition of proliferation and induction of apoptosis.

The mechanism by which changes in gene expression are thought to occur following HDAC inhibition is increased acetylation, resulting in increased recruitment of DNA-binding transcription factors, which in turn leads to increases in the expression of particular genes and decreases in the expression of other (downstream) genes. The amounts of acetylated histones (H4 or H3) increase in the presence of HDACi, as can be seen for some other acetylated proteins (e.g. p53, YY1).

The increased expression of p21 and of the luteinizing hormone receptor is only partly due to a general increase in acetylation, Sp1 sites in the promoter seem to be obligatory for reaching full induction of expression after HDACi treatment. This is possibly because HDAC1/2 can bind directly to Sp1, but Sp1 sites are also able to protect against DNA methylation, resulting in a more transcription-ready state of the promoter.

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On the other hand, the possibility of a direct response via other (unknown) DNA binding proteins that use HDACi as a ligand cannot be ruled out as yet.

By studying the effects of HDACi on the expression of various genes and their regulatory pathways, a more detailed picture will emerge of how the inhibition of HDACs, combined with the HDAC expression profile of that cell, ultimately determines the fate of the cell.

Since it is not always entirely clear which sequence of events has culminated in the remission of disease in one patient and not in others, the results of in vitro studies will be indispensable for developing a better understanding of the mechanisms involved.

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**RESPONSIVE GENES**

Listed are genes whose expression has been shown to be affected by HDACi. No discrimination is made between increased and decreased expression. Abbreviations: RAR, retinoic acid receptor; MMP, matrix metalloproteinase; PKC, protein kinase C; HSP, heat-shock protein; ICAM, intercellular cell-adhesion molecule; HES, hairy and enhancer of split; hTERT, human telomerase reverse transcriptase; IL, interleukin; VEGF, vascular endothelial growth factor; CPA3, carboxypeptidase A3.

**Function** | **Genes**
---|---
Cell cycle | Cyclin A, cyclin E, cyclin B1, cyclins D1 and D3, p21^{WAF1/Cip1}, p53
Apoptosis | CD95/CD95, gelsolin,Bax, Bcl-2
Transcription factors | GATA-2, c-Myc, RARα and β
Other | ErbB1, erbB2, Raf-1, MMP-2, tob-1, p55 Cdc, PKCδ, HM89, HSP70, ICAM-1, STRA13 (related to HES genes), hTERT, IL-6 receptor, IL-2, -8 and -10, VEGF, Notch, CPA3
**Myc oncogene**

Although myc was among the very earliest oncogenes identified and the subject of intense study, it has nonetheless proven to be an enduring enigma.

To a large extent the problem derives from the apparent gap between Myc's biological role and what is surmised to be its molecular function.

Myc family proteins (comprising c-, N-, and L-Myc) promote proliferation, growth, and apoptosis; inhibit terminal differentiation; and, when deregulated, are profoundly involved in the genesis of an extraordinarily wide range of cancers.

Alongside this veritable mountain of biological effects, the molecular characterization of Myc as a relatively weak transcriptional regulator of uncertain target genes looks like a molehill. Indeed some have wondered whether the transcriptional activities of Myc might be more apparent than real.

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**Myc oncogene**

Myc-Max heterodimers recognize the E-box sequence CACGTG (as well as related non-canonical sites) and activate transcription from synthetic reporter genes containing multimerized binding sites in mammalian cells as well as yeast

Myc and Mad mediate histone acetylation and deacetylation via the association of Mad proteins with HDACs, and of Myc proteins with HATs.

A recent study indicated that no augmentation of histone acetylation can be detected upon Myc binding to the promoters of the CAD and telomerase genes.

The binding of the target gene E-boxes by Myc correlates well with increased acetylation of histone H4.
The paradigm for local remodeling of chromatin through gene-specific recruitment of HAT activity is the retinoic acid receptor (RAR).

RAR-mediated chromatin remodeling is perturbed in acute promyelocytic leukemia (APL).

**RAR is a ligand-dependent transcriptional activator.**

- Through its zinc (Zn) finger domain, it binds as a heterodimer with a related protein, RXR, to a well-defined consensus DNA sequence found in the promoters of retinoic acid-responsive genes.

- In the absence of ligand (retinoic acid [RA]), the RXR/RAR heterodimer binds to DNA and actively represses transcription below the basal level expected from random initiation by the transcriptional machinery.
An indirect mechanism

The RXR/RAR heterodimer binds a nuclear corepressor molecule, either N-CoR (Nuclear Receptor Co-Repressor) or SMRT (Silencing Mediator of Retinoid and Thyroid Receptors), through specific interaction domains in the ligand-binding region of RAR. N-CoR, the better analyzed of the 2, binds to many sequence-specific DNA-binding transcriptional repressor proteins. N-CoR (or SMRT) itself binds another intermediary protein, Sin3, which serves as a bridge to HDAC1, a histone deacetylase.

Thus, the end result of the association of unliganded RAR/RXR with N-CoR is to recruit HDAC1 to the local environment of the promoter. By removing acetyl groups from histones and restructuring the chromatin into a repressive configuration, HDAC1 serves as the effector molecule in this pathway. N-CoR, Sin3, and HDAC1 function in many repressive pathways, including transcriptional silencing by the MAD/MAX members of the MYC family, ETO and other members of the steroid hormone receptor superfamily.

2/9/2006

CHROMATIN REMODELING MECHANISMS IN LEUKEMIA

APL: Abnormal histone deacetylation.

Chromatin remodeling is fundamental to transcription. Disruption of these mechanisms gives rise to transcriptional chaos and leukemic transformation. The best understood example of this is in APL (French-American-British [FAB] M3).

APL holds a unique position in the study of leukemias in that it is the only form of leukemia and the only malignancy described to date that responds to differentiation therapy.

APL blasts undergo terminal differentiation in response to all-trans retinoic acid (ATRA). Differentiation therapy with ATRA has become the mainstay of therapy for this disease. Although relapses uniformly occur when used by itself, in combination with conventional chemotherapy, ATRA has revolutionized the treatment of APL, generating response rates of close to 90%, with 3-year disease-free survival greater than 75%.

The explanation for the restriction of the success of ATRA therapy to the M3 subtype of leukemias likely lies in the chromatin alterations induced by the RAR-fusion proteins expressed uniquely in APL cells.

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Fusion proteins in acute promyelocytic leukemia

(A) indicates the unliganded interactions of the RXR/RAR heterodimer with an N-CoR/Sin3/HDAC1 complex. Upon binding retinoid acid, the RXR/RAR heterodimer releases the corepressor complex and binds a coactivator complex with histone acetylase activity. (B) indicates the analogous interactions of the RXR/PML-RAR heterodimer with the corepressor complex. Release of the corepressor complex occurs only in the presence of pharmacological levels of retinoic acid. (C) depicts the ligand-independent binding of the corepressor complex to PLZF-RAR. (It has been proposed, but not yet been formally demonstrated, that liganded RXR/PLZF-RAR binds both coactivator and corepressor complexes.) Chromatin remodeling occurs only in the presence of both RA and an HDAC inhibitor.

MLL fusions: A SET of alterations.

The MLL locus is involved in a greater assortment of chromosomal rearrangements in leukemias than any other gene.

Translocations or inversions of this gene on chromosome 11q23 are associated with a variety of FAB subtypes and some lymphoid malignancies as well (thus the name, Mixed Lineage Leukemia).

MLL rearrangement is found in both de novo leukemias and chemotherapy-associated (most often topoisomerase-inhibitor) secondary leukemias.

All of the 11q23 leukemias are aggressive, respond poorly to chemotherapy, and have a poor prognosis.

More than 40 translocation sites have been identified for MLL, and nearly 20 fusion partners have been cloned.

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### MLL Fusion Proteins That May Affect Chromatin Remodeling

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Karyotype</th>
<th>Potential Target Pathway</th>
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<tbody>
<tr>
<td>All MLL-fusions</td>
<td>Any 11q23 abnormality</td>
<td>SWI/SNF</td>
</tr>
<tr>
<td>MLL-AF9</td>
<td>t(9;11)(p22;q23)</td>
<td>SWI/SNF</td>
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<tr>
<td>MLL-ENL</td>
<td>t(11;19)(q23;p13.3)</td>
<td>SWI/SNF and/or HAT</td>
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<tr>
<td>MLL-CBP</td>
<td>t(11;16)(q23;p13)</td>
<td>HAT</td>
</tr>
<tr>
<td>MLL-p300</td>
<td>t(11;23)(q23;q13)</td>
<td>HAT</td>
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### CHROMATIN THERAPY

Inhibition of histone deacetylase, in part because HDAC1 was one of the first enzymes identified in nucleosomal remodeling, because its function is best understood, and because it is the only candidate for which specific inhibitors have been identified.

**HDAC inhibitors are important part of leukemia treatments.**

The clinical use of HDAC inhibitors need not be limited to patients with obvious abnormalities in histone deacetylation pathways. In 1983, continuous infusion of butyrate, at a dose of 500 mg/kg/d, was reported to induce a partial remission in a patient with myelomonocytic leukemia.

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It is currently thought that many HDACi function by blocking access to the active site (reversible or irreversible) of HDAC. There are many inhibitors known, but the most potent discovered so far is TSA.

TSA is a fermentation product of *Streptomyces*. Originally TSA was used as an anti-fungal agent, but later it was discovered to have potent proliferation-inhibitory properties with cancer cells. TSA belongs to the group of hydroxamic acids, and is effective at nanomolar concentrations *in vitro*.

The ability of TSA to inhibit HDAC was reported for the first time by Yoshida et al. in 1990. Because the production of TSA is costly and highly inefficient (20 steps, resulting in a 2% yield), the search for alternative HDACi is ongoing and of high importance.
Some of the compounds mentioned above have been entered into clinical trials [butyrate, phenylbutyrate, depsipeptide, pyroxamide, suberoyl anilide bishydroxamide (SAHA), valproic acid, CI-994].

In many studies, an increase in histone acetylation has been observed; this resulted in a partial response in some patients, but in a few patients a complete response was achieved.

The proposed mechanism consists of the re-expression of silenced genes and/or the silencing of downstream genes due to the regained access of their promoters to other modulatory factors.

The intriguing results achieved in these clinical trials might contribute greatly to enhancing our understanding of HDAC function, although little is known as yet about the specific functions of the individual HDACs and the specific consequences of using HDACi in normal cells.

Therefore studies investigating the mechanism of action of all HDACs, plus the global effects of using HDACi, are warranted.

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References:


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