Epigenetics in Hematological Malignancies

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What is Epigenetics?

The prefix **'epi'** is derived from the Greek preposition 'nope' meaning: **'above'.** In biology, 'Epigenetics' is the study of cellular and physiological trait variations that are not caused by changes in the DNAsequence.Broadly, we can consider them semipermanentchanges that effect a set of gene expression changes; however, theyare reversible.

Epigenetic changes are primarily acquired through DNA methylation, which occurs at the cytosine located in a CpG dinucleotide (regions of DNA where a cytosine nucleotide occurs next to a guanine nucleoti

de in the linear sequence of bases along its length), and post-translational histone modifications. These chromatin modifications are usually tightly regulated in development and differentiation. (1)

DNA methylation

The regulation and maintenance of DNA methylation isessential for appropriate embryonic development, cellular differentiationand genome stability.The catalyticactivity of a family of enzymes known as DNA methyltransferases(DNMTs) results in the addition of a methyl group tothe five-carbon position of cytosine bases in CpG dinucleotides,yielding 5-methylcytosine (5mC).DNA methylation has traditionally been thought to mediatetranscriptional silencing and the formation of repressive chromatinstates in addition to maintaining gene expression patternsthrough mitotic cell division. (2)

Disruption of methylation profiles and genome wide loss ofepigenetic stability is observed in malignant transformation.Although aberrant hypermethylation and silencing of tumorsuppressor genes has been found in almost all forms of cancer,both hypomethylation and hypermethylation of promoterCpG islands can affect the expression of protein coding genesand non-coding RNAs resulting in tumorigenesis.(3)

Thesechanges are highly disease specific with distinctive methylationpatterns able to distinguish between hematologic malignanciesand even subtypes of these malignancies. (4)

Using emerging high throughput DNA sequencing techniques,recurrent DNMT3A mutations were identified inapproximately 20% of patients with AML. DNMT3Amutations are enriched in cytogenetically normal, intermediaterisk AML and commonly co-occur with mutations inFms-Related Tyrosine Kinase 3 (FLT3), Nucleophosmin 1(NPM1) and isocitrate dehydrogenase (IDH)1/2. (5)

DNMT3Amutations have also been identified inpatients with myelodysplastic syndromes (MDS) andmyeloproliferative neoplasms (MPN), and are associatedwith increased likelihood of progression to AML. (6, 7)

DNA methylation as a therapeutic target in myeloidmalignancies

Emerging therapeutic strategies targeting epigeneticmechanisms of disease have shown significant promisewith the establishment of DNMT inhibitors as a cornerstoneof management in MDS. DNMT inhibitors such as5-azacitadine and 5-aza-2'-deoxycytidine are nucleosideanalogs that covalently trap DNMT1 following incorporationinto DNA resulting in genome-wide hypomethylationthrough passive dilution of 5mC. (8)

The hypomethylating effects of these agents are at noncytotoxicdose ranges limiting the severity of side effects.Further developmentof the treatment paradigm has suggested that less toxic regimens(lower doses with more frequent dosing) and the useof maintenance DMNT inhibitors as adjunct therapy or incombination with other novel therapies such as lenalidomidemay be effective in subsets of patients with high-riskMDS/AML. (9)

DNA hydroxy-methylation and the TET enzymes

Though DNA methylation was initially believed to be arelatively stable DNA modification, genome-wide high resolutionmapping of 5mC during cellular differentiation andthe recent identification of the Ten-Eleven-Translocation(TET) enzymes has revealed a more dynamic state ofaffairs. The three TET enzymes (TET1-3) catalyze the successive oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxycytosine. (10)

The 5mC derivativeshave beenshown to act as essential intermediates in both active andpassive DNA demethylation, to modulate the binding andrecruitment of chromatin regulators including the polycombrepressive complexes (PRC), and are involved in the reversal of transcriptional silencing. (11)

TET2 hasbeen shown to be mutated in myeloid malignancies includingAML, MDS and MPN with a high proportion of patientswith MDS and chronic myelomonocytic leukemia (CMML)harboring mutations. (12)

TET2 mutations are enriched inpatients presenting with a normal karyotype, is associatedwith poorer OS in AML and CMML but is not predictiveregarding clinical outcome in MDS and MPN. AlthoughTET2 mutations do not have a strong predictive correlationwith clinical outcome in MDS, TET2 mutations may independentlyact as a biomarker for response to hypomethylatingagents. (13, 14)

Histone modifications

The post-translational modification of histone tails by chromatin modifying enzymes has significant impact on intra- and inter-nucleosomal interactions. A considerable number of histone residues can be modified and the diversity of modifications result in highly complex and orchestrated chromatin environments that are dynamically altered in specific cellular contexts. These modifications not onlyhave the ability to regulate the binding of effector moleculesessential to DNA processes including transcription, repairand replication, but also the ability to regulate higher orderchromatin structure and stability.Therefore it is not surprisingthat many chromatin modifying enzymes arederanged during malignant transformation. (15)

Critical protein-protein interactions and essential co-factorsfor enzymatic activity have been identified as viabletherapeutic targets and demonstrate significant promise inthe treatment of malignancies arising from abnormalities inepigenetic regulation. (16)

Acetylation

Histone acetylation, one of the best studied histone modifications,is dynamically controlled by two opposing familiesof enzymes: lysine acetyltransferases (KATs) and histonedeacetylases (HDACs).KATs are subdivided on the basis of intracellular localizationinto predominantly nuclear (type A) or cytoplasmic (type B)subtypes. Enzymes found in the CBP/p300, MYST andGNAT families are type A KATs. (17)

Recurrent mutations in CBP and p300 are noted in a rangeof hematologic malignancies, especially the lymphoid neo-plasms. Similarly, chromosomal translocations involvingKATs (e.g. MLL-CBPand MOZ-TIF2) are found inmyeloid malignancies. (18, 19, 20)

In general, therapeutic targeting of KATs has thus far beenhampered by their low substrate specificity and broadinvolvement in multi-protein complexes that define theirmolecular activity. Interestingly, a recent structure based insilicoapproach has identified a commercially available, smallmolecule p300/CBP inhibitor; C646. C646 resulted inselective in vitro inhibition of primary human AML bearingthe AML1-ETO translocation through cell cycle arrest andapoptosis. This was associated with a dose-dependentreduction in global histone H3 acetylation and decreasedexpression of c-kit and bcl-2. (21)

Recurrent mutations of HDAC's are notobserved in cancer genomes yet HDAC inhibitors havebroadly been trialed in a range of malignancies.This is primarilybecause

they are aberrantly recruited by variousoncoproteins to inappropriately initiate or maintain malignantgene expression programs. (22)

For instance, the leukemicfusion proteins PML-RARα and PZLF-RARα have beenshown to recruit HDAC containing repressor complexesresulting in aberrant gene silencing. In murine models ofAPML, the use of HDAC inhibitors (HDACi) is effective inpotentiating or restoring the retinoid-induced differentiation of retinoic acid sensitive and resistant tumors resultingin improved survival. (22, 23)

The efficacy of HDACi in the treatment of cutaneous Tcelllymphoma has been established. However, the broaderapplication of this class of therapies in other hematologicmalignancies is yet to be clinically proven.Although initially regarded as straightforward activatorsof transcription through direct histone hyperacetylation,a greater appreciation of the non-histone effects ofHDACi on proteins such as p53 and key members of theproteasome/aggresome pathways, HSP90 and tubulin haveemerged. (24, 25)

Recent mechanistic insight into the antileukemicactivity of HDACi in t(8;21) AML demonstratesthe induction of terminal myeloid differentiation followingHDACi mediated proteasomal degradation of theAML1/ETO9a fusion protein. (26)

Over 40bromodomain containing proteins in eight subfamilies withfunctionally diverse roles such as chromatin remodeling,post-translational histone modificationand transcriptionalco-activation have been identified.For example, highly specific small molecule inhibitors targetingthe protein-protein interactions of the Bromodomainand Extra Terminal (BET) proteins (BRD2, BRD3, BRD4 andBRDt) have emerged as promising therapeutic avenues ininflammation and cancer.Pharmacological BET inhibitionshows remarkable efficacy in vitro and in vivo against MLLfusion leukemia through rapid induction of cell cycle arrestand apoptosis. (27)

Broader extension of pharmacological BET inhibition toother genetically distinct AML subgroups results in theidentification of a core transcriptional program includingcritical oncogenic targets such as BCL2 and C-MYC.

The efficacy of BET inhibition has been replicated in abroad range of hematologic malignancies including multiplemyeloma, non-Hodgkin lymphoma and ALL.These serve as proof of principle for epigenetic targetedtherapies directed against protein-protein interactions, andhave formed the basis for the initiation of early phase clinicaltrials. (28)

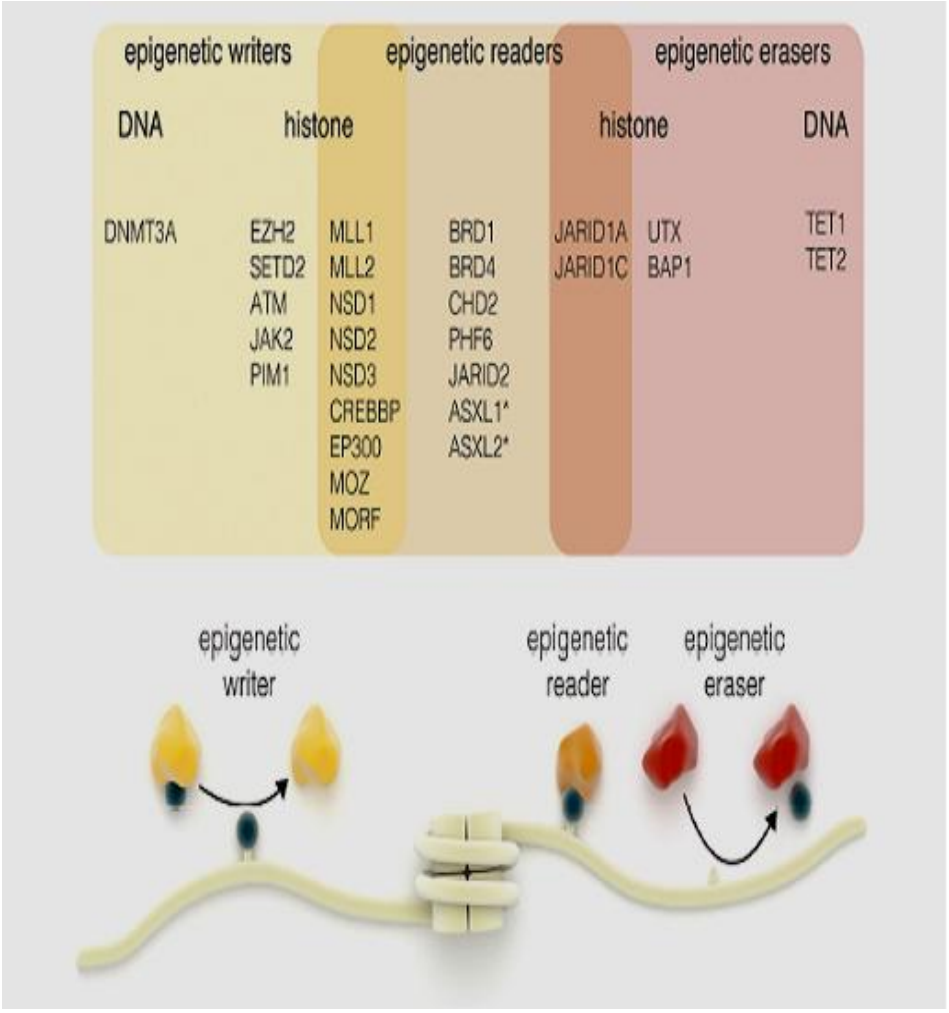

Figure 1:Epigenetic writers, readers and erasers mutated or translocated in hematologic malignancies. Epigenetic writers catalyze the chemical modifications of amino acids on histones or the cytosine base of DNA. Epigenetic erasers catalyze the removal of these modifications and epigenetic readers recognize these modifications and recruit larger macromolecular complexes to the chromatin template. A number of epigenetic writer and erasers also have domains that allow them to function as epigenetic readers (highlighted in the overlap shaded areas).

*ASXL1 and ASXL2 have a PHD domain that may allow them to function as epigenetic readers; however, there is still no conclusive evidence for this.

Methylation

Histone methylation occurs predominantly on lysine andarginine residues and is mediated by lysine methyltransferases(KMTs) and protein arginine methyltransferases (PRMTs). (29)

The functional impact of histone methylation is contextualand can lead to both transcriptional activation andrepression.The best-characterized sites of histone lysinemethylation include H3K4, H3K9, H3K27, H3K36, H3K79and H4K20.(30)

Adding to the complexity, themethylation state of individual histone residues also influencesfunctional relevance. For example, monomethylationof H3K9 is associated with active transcription whereastrimethylation is associated with repression116 and, whilstH3K4me2/3 is associated with TSS of active genes,H3K4me1 is associated with active enhancers. (31)

MLL leukemia as a model for therapeutic targetingof disordered epigenetic regulation

Wild-type MLL (WT-MLL) plays an integral role in normalembryogenesis and hematopoiesis. It is a 430 kDaprotein post-translationally cleaved into N-terminal and C-terminalfragments which re-associate to form the MLLcomplex. The C-terminal fragment contains a SETdomain, which methylates H3K4. WT-MLL also has 3HMG-like AT hooks that bind AT rich DNA; a CxxCdomain, four Plant Homeo-Domain (PHD) fingers, a bromodomain,host cell factor binding motif and transactivationdomain mediate interactions with several protein complexes(Figure 2A). (32)

Translocations involving this essential epigenetic regulatoraccount for the vast majority of infantile and approximately10% of adult leukemias.MLLleukemias follow anaggressive clinical course with poor response to conventionalchemotherapy and frequent early relapse.(33)

Demethylation

Analogous to DNA methylation, the discovery ofenzymes capable of reversing lysine methylation has highlightedthe dynamic nature of histone modifications.

In cell line models ofsubtypes of AML, pharmacological inhibition ofKDM1A in combination with ATRA results in reactivationof ATRA-dependent differentiation pathways.Theseeffects were associated with gene-specific, selective increasesin H3K4me2 and were respectively associated withdownregulation of genes bound by MLL-FP and upregulationof genes associated with myeloid differentiation. 176

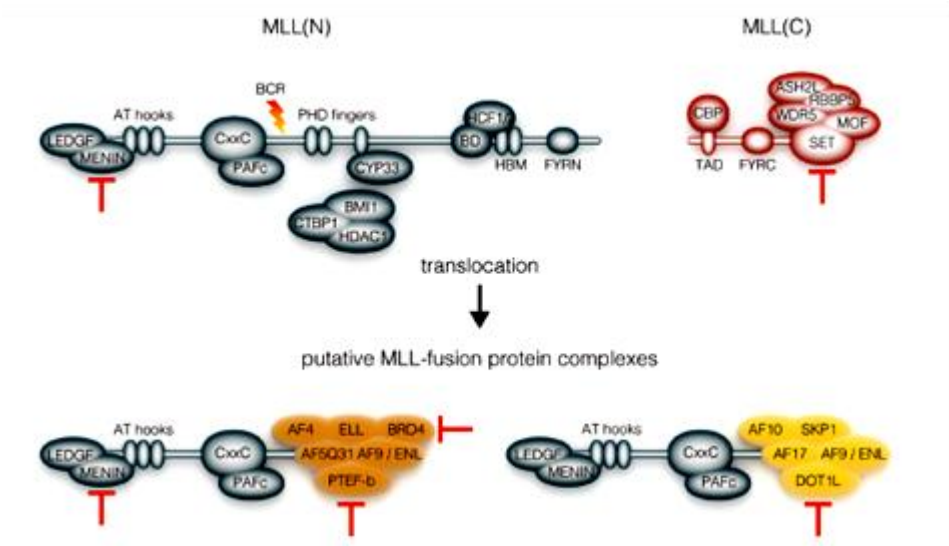

Figure 2:-MLL fusion proteins as targets for small molecule inhibition. Schematic diagram of wild-type MLL illustrating the various specialized domains and the protein-protein interactions mediated by them. Also illustrated are the purported MLL-fusion protein complexes. Following translocation, a fragment of the N-terminal portion of MLL is fused in frame with a translocation partner leading to the formation of novel MLL-fusion protein complexes including the SEC and DOT1L complex. It is unclear whether these are separate entities or co-exist as one large complex. Highlighted are various small molecules that have been developed to target the leukemogenic capacity of either wild-type MLL or MLL-fusion proteins. BCR: breakpoint cluster region; HBM: host cell factor binding motif; TAD: transactivation domain.

Phosphorylation

Kinases and phosphatases control the addition andremoval of phosphate groups on serine, threonine and tyrosineresidues of component histone proteins. Histone phosphorylation results in gross changes inchromatin structure and has been implicated in the regulationof gene transcription, DNA repair and chromatin condensation.Aberrant kinase activity is one of the most commonlyobserved processes in malignant transformation. (34)

Constitutive activation of JAK2, a non-receptor tyrosinekinase crucial for cytokine signaling in normalhematopoiesis, commonly occurs in MPN.The identification of multiple pathogenic consequencesof aberrant signaling kinase activity at chromatin broadensthe therapeutic scope of kinase inhibitors currently in clinicaldevelopment. Several kinase inhibitors result in globalreduction of histone modification laid down by targetenzymes (e.g. JAK2 and Aurora kinase inhibitors) and thuscan be considered as potential epigenetic therapies. (35)

Table 1. Current development of targeted epigenetic therapies.

	Target enzyme	Disease type	Current stage of development
Writers			
Acetylation	CBP/p300	AML, Ovarian, Colon, Melanoma	Pre-clinical
	PCAF	Ovarian, Colon	Pre-clinical
Methylation	DOT1L	MLL-r leukaemia	Clinical
	EZH2	NHL, advanced solid tumors	Clinical
Phosphorylation	JAK2	MPN	FDA approved
	Aurora kinase	NHL, CML, ALL	Clinical
Erasers			
Acetylation	HDACi	CTCL	FDA approved
Methylation	LSD1/KDM1A	AML	Clinical
	UTX/JMJD3	Inflammatory response	Pre-clinical
Readers			
Acetylation	BET	Haematological malignancies, NUT midline carcinoma	Clinical
DNA Methylation			
	DNMT	MDS	FDA approved
	IDH inhibitors	AML, glioblastoma	Clinical

MLL-r: mixed lineage leukemia rearranged; NHL: non-Hodgkin lymphoma; MPN: myeloproliferative neoplasms; CML: chronic myeloid leukemia; ALL: acute lymphoblastic leukemia; CTCL: cutaneous Tcell lymphoma; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome.

Conclusions

Although highly heterogeneousin nature, aberrant regulation of epigenetic processes hasemerged as a prominent unifying theme in hematologicmalignancies.

Somatic alterations of epigenetic regulatorssuch as *DNMT3A*, *TET2*,*IDH2*, *MLL*, *EZH2* and*ASXL1* have prospective prognostic value in AML andMDS.

Therapies directed against epigenetic mechanismsof disease have also entered widespread clinical practicewith resultant improvement in clinical outcomes.

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