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DNMT3A in haematological malignancies

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Abstract

DNA methylation patterns are disrupted in various malignancies, suggesting a role in the development of cancer, but genetic aberrations directly linking the DNA methylation machinery to malignancies were rarely observed, so this association remained largely correlative. Recently, however, mutations in the gene encoding DNA methyltransferase 3A (DNMT3A) were reported in patients with acute myeloid leukaemia (AML), and subsequently in patients with various other haematological malignancies, pointing to DNMT3A as a critically important new tumour suppressor. Here, we review the clinical findings related to DNMT3A, tie these data to insights from basic science studies conducted over the past 20 years and present a roadmap for future research that should advance the agenda for new therapeutic strategies.

Previous interest in DNA methylation in cancer focused on the role of DNA methyltransferase 1 (DNMT1) because mutations in this protein have been described in colorectal, prostate and haematological malignancies, albeit rarely[1], and reduced DNMT1 activity has been shown to promote cancer in mouse models[2,3]. Recent cancer genome sequencing efforts exposed *DNMT3A* as one of the most frequently mutated genes across a range of haematological malignancies, raising questions concerning how these lesions promote malignancies. Basic research has examined the role of DNMT3A in gene repression, but the insights gained from these fundamental studies have not been placed in context with the recent findings suggesting that *DNMT3A* mutations play a prominent part in clonal and malignant haematopoiesis. The intention of this Review is to analyse the clinical findings related to DNMT3A and survey its role in normal stem cell biology, highlighting the large conceptual gaps that remain to be filled to understand and target *DNMT3A* mutation-associated malignancies. Although DNMT3A has been implicated in

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DATABASES
COSMIC: http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/

SUPPLEMENTARY INFORMATION
See online article: S1 (table) | S2 (table)

several types of cancer[4–6], we focus primarily on the role of DNMT3A in haematological malignancies.

DNA methylation

DNA methylation is an epigenetic modification that is important in development, imprinting, stem cell regulation, X-chromosome inactivation and several diseases[7]. Methylation of DNA refers to the addition of a methyl (CH_3) group to the C5 position of the pyrimidine ring of cytosines to form 5-methylcytosine (5mC)[8], usually in the context of a CpG dinucleotide pair. Although 60–80% of individual CpGs are methylated, clusters called CpG islands in gene regulatory regions tend to be unmethylated. In general, high DNA methylation is associated with the silencing of gene expression[9]. DNA methylation is particularly concentrated on repetitive elements, and it may limit their genomic activity.

In cancer, aberrant DNA methylation has been observed for more than two decades, with interest initially focused on promoter hypermethylation and the consequent silencing of tumour suppressor genes[10,11]. Global hypomethylation was also observed and proposed to be associated with genomic instability[12]. Regions of lower-density methylation near CpG islands, known as ‘shores’, exhibit great variation in methylation, including hypomethylation and hypermethylation, across different types of cancers[13]. Importantly, the mechanisms that drive aberrant methylation and its pathological importance are mostly unknown.

DNA methylation is mediated by a family of DNA methyltransferase enzymes, including DNMT1, DNMT3A and DNMT3B[14]. The related member DNMT3-like (DNMT3L) lacks a catalytic domain and functions as an accessory protein to DNMT3A during embryonic development and genomic imprinting[15,16]. DNMT1 primarily maintains pre-existing DNA methylation patterns[17], whereas DNMT3A and DNMT3B carry out *de novo* DNA methylation[14]. DNMT2 is considered an RNA methyltransferase[18]. The methylcytosine dioxygenase proteins (TET1, TET2 and TET3), convert 5mC to 5-hydroxymethylcytosine (5hmC)[19]. 5hmC is not maintained by DNMT1 but the mark leads to passive demethylation during cell division[20,21] and thus provides a mechanism for DNA demethylation.

Structure of DNMT3A

DNMT3A is a 130 kDa protein encoded by 23 exons on human chromosome 2p23 (REF. 22). The protein is highly conserved across mammals, with 98% homology between human and murine homologues[23]. There are two major splice isoforms of the murine RNA: *Dnmt3a1* (long) and *Dnmt3a2* (short). *Dnmt3a2* lacks the first six exons of the amino-terminal domain[24], and its expression is restricted to embryonic stem cells (ESCs), testes, ovaries, spleen and thymus[24,25]. *Dnmt3a1* is widely expressed and transcribed from two different promoter regions[11,26]. The basis for the cell type specificity and significance of the isoforms is not known.

The major protein domains are the Pro-Trp-Trp-Pro (PWWP) domain, the ATRX-DNMT3-DNMT3L (ADD) domain (also known as the plant homeodomain (PHD)) and the catalytic methyltransferase domain (FIG. 1). Although they have not been exhaustively studied, the

ADD and PWWP domains are known to interact with proteins that are involved in transcriptional repression, and the N terminus is thought to be involved in DNA binding[27–31]. The ADD domain also inhibits the DNMT3A catalytic domain by forming an auto-inhibitory loop that is released by interaction with the unmodified lysine 4 of histone H3 (H3K4me0), linking DNMT3A and H3 chromatin marks[32].

The catalytic domain of DNMT3A is highly conserved, even within prokaryotic DNA methyltransferases[33], implying that the specificity of DNMT3A originates in its regulatory domains. X-ray crystallography indicates that the murine protein acts as a heterotetramer, with the catalytic core consisting of a mixed β-sheet of seven strands, among which the methyl donor and DNA binding functions are distinguished[34]. Interaction with DNMT3L substantially increases enzyme processivity[15,35]. DNMT3B, which is usually co-expressed with DNMT3A, is expressed in multiple isoforms with different activities[36,37], some of which may also influence DNMT3A activity, particularly in cells that lack DNMT3L[38].

Methylation activities of DNMT3A

DNMT3A is reported to methylate DNA at unique sites as well as at repetitive elements. In mouse ESCs, DNMT3A localizes to discrete nuclear foci and, together with DNMT3B, to `pericentromeric heterochromatin`[39], suggesting that the DNMT3A–DNMT3B dimer methylates major satellite repeats at these sites[40]. Furthermore, in *Dnmt3a* and *Dnmt3b* double-knockout (*Dnmt3a*$^{-/-}$*Dnmt3b*$^{-/-}$) ESCs, transfection of *Dnmt3a* restores methylation at specific repetitive element families[36]. In genomic studies, DNMT3A methylates specific regions of the genome overlapping with those methylated by DNMT3B[36,41,42], suggesting some degree of functional redundancy. DNMT3A also methylates free or linker DNA with higher efficiency than nucleosome-bound DNA[43,44].

Some of the gene-silencing activities of DNMT3A are intimately tied to chromatin modifications through interactions with specific proteins (FIG. 1). As described above, DNMT3A interacts with H3K4me0, a mark of inactive gene transcription[30,32]. DNMT3A also interacts with histone modifiers involved in gene repression, such as the histonelysine *N*-methyltransferases SUV39H1, SETDB1 and G9A (also known as EHMT2)[45–51], which are linked to H3K9 methylation. Similarly, the histonelysine *N*-methyltransferase enhancer of zeste homologue 2 (EZH2), which is the catalytic component of Polycomb repressive complex 2 (PRC2), is required for DNA methylation of EZH2 target promoters and interacts with DNMT3A, DNMT3B and DNMT1. This interaction, however, is insufficient for *de novo* DNA methylation, suggesting that other factors are needed to initiate methyltransferase activity[52,53]. Overall, most studies have been limited to specific cells and loci, and we still have a poor understanding of how DNMT3A activity is regulated more broadly. How is DNMT3A recruited to chromatin? How does it target particular sites? And how is the protein regulated through interactions with other protein partners? These are crucial questions that need to be answered if we are to understand cancer development and to develop targeted therapies.

Unique methylation patterns set by DNMT3A have also been investigated. In haematopoietic stem cells (HSCs), DNMT3A probably regulates methylation at the edges of

large regions of hypomethylation called DNA methylation canyons[54] (or valleys[55]). DNMT3A is also involved in gene body DNA methylation[56].

Although there is no known consensus sequence that DNMT3A methylates[57], biochemical studies show that DNMT3A prefers to methylate sequences rich in T, C and A around CpG sites, methylating one strand at a time[15,58–60]. Methylation at CpGs is the primary target, but DNMT3A is also active in other contexts, such as CpA[61–63] (also termed CpH methylation, where H can be A, C or T). This non-CpG methylation occurs at extremely low levels in most tissues, except in ESCs and the postnatal brain[64–66], and its potential function is a matter of controversy[67]. The relative importance of different DNA methylation sites, and how DNMT3A activity is directed to different types of loci, is unclear. These multiple targets of DNMT3A underscore the complexity of its functions and the major gaps in our understanding of its regulation.

Role of DNMT3A in development

Mouse models have offered important insights into the function of the DNMT family. *Dnmt3a*$^{-/-}$ mice survive for approximately 1 month after birth but die with an uncharacterized failure to thrive, whereas *Dnmt3b*$^{-/-}$ mice die mid-gestation. *Dnmt3a*$^{-/-}$*Dnmt3b*$^{-/-}$ embryos show limited embryonic development[68]. Both *Dnmt3a* and *Dnmt3b* are highly expressed in mouse ESCs and are downregulated during differentiation[14]. Although deleting either gene alone has minimal impact, eliminating both together in ESCs resulted in persistent self-renewal and inefficient differentiation, as measured by `teratoma formation assay` after serial passage[36]. This phenotype was associated with DNA hypomethylation[36] and incomplete repression of pluripotency genes after differentiation[69].

Recently, *Dnmt3a* was also found to have a role in somatic stem cell differentiation. When *Dnmt3a* was conditionally deleted in HSCs, self-renewal was markedly favoured over differentiation. This increased self-renewal caused *Dnmt3a*-null HSCs dramatically to outcompete their wild-type counterparts and accumulate in the bone marrow[70]. The *Dnmt3a*-null HSCs exhibited a surface phenotype that was indistinguishable from that of their wild-type counterparts, and they differentiated into all types of downstream progeny, such as myeloid cells, B cells and T cells; however, the output of differentiated cells per HSC was reduced relative to that of wild-type cells[70]. Similar to wild-type HSCs, they were mostly quiescent, suggesting that their competitive advantage lay in the proportion of cell divisions that generated stem cells versus differentiated cells. When *Dnmt3b* was ablated, HSCs showed only minor differences from wild-type cells, perhaps because the predominant splice isoform generates a catalytically inactive protein. However, its ablation along with *Dnmt3a* (*Dnmt3a*$^{-/-}$*Dnmt3b*$^{-/-}$ HSCs) greatly exacerbated the stem cell expansion and led to almost completely blocked differentiation[71].

To date, the mechanisms accounting for increased self-renewal in the absence of DNMT3A have been studied by analysing changes in gene expression and DNA methylation in mutant cells. *Dnmt3a*-null HSCs exhibit a net loss of DNA methylation, particularly at the edges of large hypomethylated canyon regions[54], which are enriched for genes associated with stem cell self-renewal and cancer, such as homeobox A9 (*Hoxa9*), Meis homeobox 1 (*Meis1*), and

MDS1 and EVI1 complex locus (*Mecom*; which encodes the transcriptional regulator ecotropic virus integration site 1 protein homologue (EVI1)). In *Dnmt3a*$^{-/-}$ mice, many genes associated with HSC self-renewal increase in expression, and some fail to be appropriately repressed during differentiatiation[70]. These data suggest that the absence of DNMT3A abrogates the ability to switch from a self-renewal to a differentiation programme, ultimately resulting in more self-renewal cell divisions.

Analysis of the mechanisms leading to the differentiation block of *Dnmt3a*$^{-/-}$*Dnmt3b*$^{-/-}$ HSCs suggested a contribution of β-catenin to this phenotype. The promoter region of *Ctnnb1* (which encodes β-catenin) became hypomethylated, and both gene and target gene expression increased. Knockdown of *Ctnnb1* partially rescued the differentiation block, consistent with a role for β-catenin in HSC self-renewal[71], similar to its role in ESC self-renewal[72]. However, other unknown factors are also likely to contribute.

Together, the sustained expression of self-renewal-associated genes is consistent with the expansion of *Dnmt3a*$^{-/-}$ and *Dnmt3a*$^{-/-}$*Dnmt3b*$^{-/-}$ HSCs, but many questions remain to be answered. For example, the mechanisms that dictate the activity of DNMT3A at specific genomic loci are obscure. Similarly, although the levels of DNA methylation change at canyons and other regions, how these changes contribute to the phenotype is unclear. More broadly, whether *de novo* DNA methylation can initiate or merely enforce differentiation decisions is unknown, as is a possible role for DNMT3A in maintaining ‘stemness’ beyond differentiation.

The role of *de novo* DNA methylation in the function of ESCs and HSCs raises the possibility that DNMT3A and/or DNMT3B may be involved in the differentiation of other somatic stem cell types. Indeed, DNMT3A has been implicated in neural stem cell differentiation[56,73]. In addition, germline mutations in *DNMT3A* cause a human overgrowth syndrome resulting in extreme height[74], perhaps through the modulation of tissue-resident stem cell activity.

DNMT3A mutations in blood malignancies

Despite the long-recognized aberrant DNA methylation in cancer, the first mutations in *DNMT3A* associated with malignancy were only identified in 2010, with three groups reporting mutations in acute myeloid leukaemia (AML) with frequencies of up to 22%[75–77]. A mutational hotspot at arginine 882 (R882) was highlighted, but other mutations throughout the gene were also seen. Mutations in *DNMT3A* have now been found in most types of haematological malignancy with varying frequency (TABLE 1; Supplementary information S1 (table)). Although a few mutations in *DNMT1* and *DNMT3B* have also been reported[78,79], the overwhelming prevalence of *DNMT3A* mutations across a range of diseases demonstrates the special role of this gene in preventing malignancy.

Across haematological malignancies, *DNMT3A* mutations are distributed throughout all of the functional domains (FIG. 2a), some of which have been shown by biochemical studies to govern specific functions and interactions (TABLE 2; Supplementary information S2 (table)). For example, mutation of R878 in the catalytic domain of the murine protein

(equivalent to R882 of the human protein; discussed below) has been shown to abrogate catalytic activity and to result in reduced DNA binding[60]. However, most of the specific mutations found in cancer have not been functionally characterized. Although the impact of some mutations may be predicted by the domain interactions, their specific relevance for the cancer phenotype has not been explored.

DNMT3A mutations as a pre-leukaemic lesion

Deep sequencing of haematological malignancies showed that *DNMT3A* mutations were typically found at higher `variant-allele frequencies` (VAFs) than other accompanying mutations, suggesting that they were among the first to arise[80,81]. If, as in mice, *DNMT3A*-mutant (*DNMT3A*^{mut}) human HSCs enjoy a self-renewal advantage, this could lead to their expansion over time, potentially serving as a pre-leukaemic lesion (FIG. 3). Indeed, this concept has been substantiated. Two independent studies found that human HSCs purified from patients with AML could harbour *DNMT3A* mutations in the absence of other common leukaemia-associated mutations[82,83]. The *DNMT3A* mutations also appeared in ostensibly normal lymphoid progeny from the same patients. Furthermore, as in mice, human *DNMT3A*^{mut} HSCs seemed to have a marked advantage relative to wild-type HSCs, at least in xenograft models. Together, these studies indicate that human HSCs can harbour *DNMT3A* mutations and still contribute to multiple blood lineages, existing in a pre-leukaemic state prior to the acquisition of additional mutations that lead to leukaemia.

Because HSCs are maintained *in vivo* for decades, these findings raised the possibility that *DNMT3A*^{mut} HSCs might arise months, or even years, before the development of disease. Now, extensive analysis of genome sequencing data from more than 42,000 individuals without haematological malignancies across three studies has incontrovertibly established this concept[84–86]. In multiple cohorts, haematopoiesis was shown to frequently become clonally derived with age: 5–10% of 70-year-olds derived almost all of their peripheral blood cells from a single HSC. Genome variants associated with this remarkable state were enriched for a number of cancer-associated mutations, with mutations in *DNMT3A* overwhelmingly the most common. Although most individuals harbouring such mutations did not develop haematological malignancies in the time frame examined, the somatic mutations (collectively) were associated with an increased risk of leukaemia as well as all-cause mortality[86]. This striking clonal haematopoiesis demonstrates the Darwinian nature of the bone marrow, in which a small advantage of variant stem cells can lead to their dominance over time, and highlights the particular fitness benefit conferred on stem cells by even partial loss of DNMT3A function.

These studies established the existence of preleukaemic stem cells, in which *DNMT3A* mutations are common. These mutations can predispose to, but are insufficient for, the development of leukaemia. Accordingly, these studies underscore the importance of understanding the mechanisms that endow mutant HSCs with their competitive advantage, the features that may promote or inhibit the edge that mutant HSCs have over their wild-type counterparts and the factors that influence the lag time for disease development. The observation that loss of DNMT3A in murine HSCs also confers a selective advantage, allows HSCs to persist for months in the absence of transformation[70], and predisposes to

multiple types of haematological disease[87,88] indicates that murine models may be valuable for investigating these crucial questions.

Distribution of DNMT3A mutations

Interestingly, the domains of *DNMT3A* that are enriched for mutations and the frequency of heterozygous versus homozygous (or compound heterozygous) mutations varies among different malignancies (FIG. 2). In the myeloid lineage, *DNMT3A* mutations are most prevalent in adults with AML, with most studies reporting a mutation frequency of 20–25% in *DNMT3A* in *de novo* disease[76,78,89–94]. Many studies examining all or most of the coding region in AML reported that around 60% of *DNMT3A* mutations are found at the residue R882 in the methyltransferase domain[76,92,93]. Across other myeloid malignancies, including myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPNs) and chronic myelomonocytic leukaemia (CMML), the R882 position is the most frequently mutated, although less frequently than in AML[95–100]. In all of these diseases, *DNMT3A* mutations are typically heterozygous, with biallelic involvement essentially confined to non-R882 mutants.

T lymphoid lineage malignancies also frequently harbour *DNMT3A* mutations, although the domain distribution of mutations is more diverse than that in myeloid lineages (FIG. 2). In peripheral T cell lymphoma (PTCL), *DNMT3A* mutations are clustered in the methyltransferase domain, but less than 20% affect the R882 position[101–103]. A similar proportion of mutations in T cell acute lymphoblastic leukaemia (T-ALL) affect the R882 position[96,104], and the frequency of biallelic involvement is very high, occurring in up to 62% of patients[104]. Biallelic mutation suggests a more complete loss of function, which is consistent with classic tumour suppressor activity.

The prevalence of the R882 variant has made it of special interest. Recent data indicate that R882 mutation results in a hypomorphic protein[60,105,106] that acts in a dominant-negative manner, inhibiting the methyltransferase activity of the remaining wild-type DNMT3A ($DNMT3A^{WT}$)[107,108]. In the heterozygous state, some $DNMT3A^{WT}$ function remains (estimated at 20%), either through homodimeric interactions of remaining $DNMT3A^{WT}$ or through other protein interactions that have not yet been determined[105]. By contrast, heterozygous mutation of *DNMT3A* at other sites may lower activity of the wild-type protein to only 50% and thus be insufficient to drive malignancy. Therefore, selection for a second mutation (or loss of heterozygosity) is more common in the context of non-R882 mutations. The level of DNMT3A activity in these cases remains to be examined.

The prevalence of R882 mutations in myeloid malignancies, and the predominance of biallelic mutations in lymphoid malignancies, is intriguing and not understood. We conjecture that the varying levels of residual DNMT3A activity that are present in different scenarios have distinct effects. Possibly, some DNMT3A activity is needed for myeloid lineage choice and cancer development by action at specific gene targets, whereas the development of lymphoid malignancies may tolerate (or require) more complete functional DNMT3A loss. Alternatively, interaction with accessory proteins, including DNMT3B, may vary among the lineages, leading to different outcomes. A better understanding of the

functional impact of other mutant alleles would provide valuable insight into how these malignancies develop.

The frequency of the R882 mutation may also have therapeutic implications. Patients heterozygous for the R882 mutation have some DNMT3A^{WT}, the function of which may be improved if the R882 form was selectively inhibited, whereas patients with null-like biallelic mutations may need alternative strategies to regain normal DNA methylation.

DNMT3A mutations and aberrant DNA methylation

The mechanisms through which mutations in *DNMT3A* drive leukaemia are unclear. The R882 mutation in patients with AML correlates with global hypomethylation, especially at CpG islands, shores and promoters[108,109], although some promoter hypermethylation is also reported[77,109]. Although the hypomethylation of genes previously implicated in AML, such as homeobox-containing transcription factors, can be observed[77,109], the role of these events in disease development is unknown. More large-scale and unbiased methylation studies are needed to fully assess the impact of R882 and non-R882 mutations on DNA methylation.

In *Dnmt3a*$^{-/-}$ HSCs, hypomethylation was predominantly found when whole-genome DNA methylation analysis was carried out[54]. In murine leukaemias generated from *Dnmt3a*$^{-/-}$ HSCs, loss of methylation at intergenic regions was associated with AML, whereas hypermethylation, often at CpG islands, was observed in T-ALL[87]. The mechanism that leads to an increase in methylation when a DNA methyltransferase is disrupted in mice (or mutated in humans) is enigmatic; it might originate partly from residual DNMT3B, although the predominant *DNMT3B* transcript present in samples from patients with AML[108], as well as murine HSCs[71], is thought to encode a catalytically inactive protein. Moreover, the relative contribution of hypermethylation versus hypomethylation to the pathological state and leukaemia development is not known.

Further impeding a mechanistic understanding is the relative lack of correlation between changes in DNA methylation and gene expression[56,70,110]. Emerging data from other studies suggest that changes in enhancer DNA methylation, in addition to changes in promoters, may be important[111,112]. Further studies of aberrant DNA methylation should be linked with topological studies of the genome, including the three-dimensional structure that links `enhancers` and gene expression by DNA looping[113].

The lack of understanding of how DNMT3A mutations affect DNA methylation and the poor correlation of genetic changes with gene expression have led to speculation that mutations in DNMT3A may be disrupting functions that are distinct from DNA methylation. Although no activities of DNMT3A that are unrelated to DNA methylation have been reported, this possibility certainly merits further study.

DNMT3A-related disease features

In some studies, the prevalence of *DNMT3A* mutations is associated with age, ethnicity and gender. Additionally, a number of clinical studies have reported possible associations between DNMT3A mutational status and clinical features such as blood cell counts and the

percentage of blasts in the blood and bone marrow at the time of cancer diagnosis (see Supplementary information S1 (table)). In both myeloid and lymphoid malignancies, an increased incidence of *DNMT3A* mutations with advanced age has been reported (see Supplementary information S1 (table)). This relationship with age is consistent with the recent findings of *DNMT3A* mutations linked to clonally derived haematopoiesis that is progressively more common with age[84–86]. In a subset of individuals with such clonal haematopoiesis, malignant transformation may occur after a subsequent deleterious lesion is acquired following a lag time that may be in the order of years. In accordance with this observation, *DNMT3A* mutations are exceedingly rare in paediatric haematological malignancies[114–119] (TABLE 1; see Supplementary information S1 (table)). Alternatively, fundamental differences in the pathogenic process may account for the dearth of *DNMT3A* mutations in paediatric compared to adult haematological malignancies.

Specific co-mutation patterns of DNMT3A

DNTM3A mutations occur non-randomly in association with other genetic abnormalities, including cytogenetic aberrations (FIG. 4). For example, in AML, *DNMT3A* mutations are essentially never present in patients with the chromosomal translocations t(15;17), inv(16) and t(8;21)[76,78,89,91,92,94], despite their association with specific DNA methylation patterns[78,120]. Similarly, *DNMT3A* mutations are almost never found concurrently with rearrangements involving the histone-lysine *N*-methyltransferase KMT2A (also known as MLL) in acute leukaemias, and are negatively correlated with mutations in the transcriptional regulator additional sex combs-like transcriptional regulator 1 (*ASXL1*), an enzyme that is important in H3K27 methylation in MDS[95,121,122]. Thus, the mutual exclusion of *DNMT3A* mutations and these abnormalities suggests possible convergence on similar epigenetic perturbations. Of the positive correlations, there are some striking patterns of co-mutation that seem to dictate disease outcome (FIG. 4) and that may have implications for how DNMT3A functions.

Two of the most striking examples of co-occurrence are in AML: that is, the association between *DNMT3A* mutations and mutations in the gene encoding nucleophosmin (*NPM1*), and the co-occurrence of *DNMT3A* mutations with internal tandem duplication in the gene encoding the receptor tyrosine kinase FLT3 (*FLT3*^{ITD}) (FIG. 4). A remarkable 60% of patients with *DNMT3A* mutations also carry an *NPM1* mutation, whereas only 13% of patients with *DNMT3A*^{WT} harbour an *NPM1* mutation[76,78,89,91,92,96,123,124]. Similarly, *FLT3*^{ITD} mutations are specifically enriched in patients with *DNMT3A* mutations[76,78,89,91,94,96,123,125] (FIG. 4). The known association between *NPM1* and *FLT3*^{ITD} mutations raises the possibility that the high frequency of co-occurring *FLT3*^{ITD} and *DNMT3A* mutations is merely a reflection of the high frequency of concomitant *FLT3*^{ITD} and *NPM1* mutations. However, extensive genomic and epigenomic analysis performed by The Cancer Genome Atlas Research Network found that samples with all three mutations formed distinct mRNA, microRNA and DNA methylation clusters, suggesting that the occurrence of all three mutations is non-random, and that *NPM1*^{mut}*FLT3*^{ITD}*DNMT3A*^{mut} AML is a distinct entity[78]. How these mutations interact to cause leukaemia is unclear and warrants further investigation.

In haematological malignancies, an interesting pattern exists of co-mutations of *DNMT3A* with genes encoding enzymes that are important in 5-hydroxymethylation, including isocitrate dehydrogenase 1 (IDH1), IDH2 and TET2. Mutations in *IDH1* and *IDH2* are thought to contribute to leukaemogenesis by the accumulation of 2-hydroxyglutarate, which inhibits TET2, the enzyme responsible for DNA hydroxymethylation. *IDH1* mutations are enriched in patients with AML who harbour *DNMT3A* mutations[76,78,91,124] (FIG. 4). In MDS, the correlation between mutations in *IDH1* and *IDH2* and mutations in *DNMT3A* is still uncertain; however, three of ten patients with *DNMT3A*^{mut} `secondary acute myeloid leukaemia` (sAML) derived from MDS also had mutations in *IDH1* or *IDH2*, whereas none of the patients with *DNMT3A*^{WT} sAML derived from MDS exhibited *IDH1* or *IDH2* mutations[126,127]. This suggests that the combination of *DNMT3A* mutation with mutations in *IDH1* and *IDH2* may contribute to progression from MDS to AML. The evidence suggests that an important interaction exists between mutations in *DNMT3A* and mutations in *IDH1* and *IDH2* in myeloid malignancies, implying that aberration of DNA methylation and DNA hydroxymethylation contribute to leukaemogenesis. Curiously, no specific association between *DNMT3A* and *TET2* mutations exists, perhaps suggesting an impact of *IDH* mutations on histone, rather than DNA, demethylation[128]. Nevertheless, in T cell lymphoma a strong and interesting association between mutations in *DNMT3A*, *TET2* and *IDH* exists that may have important mechanistic implications (BOX 1).

In MDS there is a strong association between *DNMT3A* mutations and mutations of the spliceosome `factor` *SF3B1* (splicing factor 3b, subunit 1), with 50–56% of patients with MDS and *DNMT3A* mutations also harbouring mutations of *SF3B1*, compared with 12–17% of patients with *DNMT3A*^{WT} (REFS 95,121,122) (FIG. 4). Co-mutation with the spliceosome factor *U2AF1* (U2 small nuclear RNA auxiliary factor 1) has also been reported[129]. By contrast, a negative correlation between mutations of serine/arginine-rich splicing factor 2 (*SRSF2*) and *DNMT3A* is seen[121], indicating differential genetic interactions between mutant *DNMT3A* and the spliceosomal machinery, and a potential mechanistic convergence (BOX 2).

Prognostic impact of *DNMT3A* mutations

Most AML studies have found no difference in the rate of complete remission between patients with and without *DNMT3A* mutations; however, analysis of the impact of *DNMT3A* mutations on survival has generated differing results. Ley *et al.* reported that *DNMT3A* mutations had a highly significant negative impact on survival[76], a finding supported by a number of large studies[90,91,93,123,124,130] but not uniformly corroborated[89,92,96]. Comparing these reports is challenging because patient populations, treatment regimens and outcome measures vary widely from study to study. The prognostic impact of R882 versus non-R882 mutations is also inconclusive[92,94,123,124].

As in AML, reports vary regarding the impact of *DNMT3A* mutations on outcome in MDS, with some studies finding a significantly worse overall and event-free survival and a higher rate of progression to AML[97,131], but others finding no significant association between outcome and mutation of *DNMT3A*[95,96,121,122,132]. Interestingly, patients with *SF3B1*^{WT} and *DNMT3A*^{mut} had an inferior overall survival and higher risk of AML transformation

compared with other related mutation combinations (*SF3B1*^{mut}*DNMT3A*^{mut}, *SF3B1*^{WT}*DNMT3A*^{WT} and *SF3B1*^{mut}*DNMT3A*^{WT})[121]. Because over 50% of patients with MDS who have *DNMT3A* mutations have a concomitant *SF3B1* mutation — and these mutations are associated with superior outcomes — perhaps the prognostic significance of *DNMT3A* mutation is diluted when only looking at these mutations as a whole group. Future studies could evaluate the prognostic impact of the *SF3B1–DNMT3A* 'risk group' to better stratify patients.

Despite the lack of clarity regarding the impact of *DNMT3A* mutation on outcome, evidence in MDS, MPN and CMML suggests that the presence of a *DNMT3A* mutation may facilitate the transition from myeloproliferation and/or myelodysplasia to frank myeloid leukaemia. In all of these neoplasms, there is a striking enrichment for *DNMT3A* mutations in sAML compared with the frequency in *de novo* acute myeloid leukaemia (*de novo* AML)[127,133,134]. In addition, the order of acquiring a mutation in *DNMT3A* relative to other genes may affect disease progression. In MDS and myelofibrosis, the *DNMT3A* mutations found in sAML can be traced back to the original MDS clone, which is consistent with the concept that *DNMT3A* mutations are likely to instigate a pre-leukaemic state. However, in the MPNs polycythaemia vera and essential thrombocythaemia, patients harbouring mutations in Janus kinase 2 (*JAK2*) may acquire *DNMT3A* mutations later[135], indicating that *DNMT3A* mutations can be acquired through the evolution of a *JAK2*^{mut} clone and that this may contribute to progression in these specific diseases.

As in myeloid diseases, a number of studies have reported significantly worse overall survival for patients with T-ALL who have *DNMT3A* mutations[96,104,136]. It is not clear whether this is cause or correlation because *DNMT3A* mutations are enriched in the more primitive and/or immature T-ALL subtypes, which tend to have a worse prognosis than mature T-ALL[104,136,137]. However, the sum of available data suggests that the presence of a *DNMT3A* mutation is a negative prognostic marker independent of disease phenotype. Thus, it is reasonable to consider screening patients with T-ALL for mutations of *DNMT3A* as a way to refine risk stratification.

Therapeutic implications

DNA-damaging anthracyclines are a key component of most AML treatment regimens. Some studies suggest that better outcomes for patients with AML who harbour *DNMT3A* mutations have correlated with intensified treatment with DNA-damaging anthracycline therapy[89,138]. If confirmed, this may indicate that AML cells with *DNMT3A* mutations are relatively resistant to this class of agents, perhaps due to *DNMT3A*-linked resistance to DNA damage-induced cell death or to other unknown mechanisms. Therefore, patients with *DNMT3A* mutations may require higher anthracycline doses compared with patients who have *DNMT3A*^{WT}. Intensification through the administration of higher daunorubicin doses or the use of a more potent anthracycline, idarubicin, may overcome the negative prognostic impact of *DNMT3A* mutations, but this strategy would come at the cost of increased toxicity; therefore, more targeted approaches are desirable.

The use of the DNA methyltransferase inhibitor 5-azacytidine and its deoxy derivative, decitabine, has become increasingly common for the treatment of MDS and is being explored for the treatment of AML. These agents are thought to work by inhibiting DNMT1, leading to the demethylation of aberrantly hypermethylated genes, such as cyclin-dependent kinase inhibitor 2B (*CDKN2B*)[139,140], *MDR1* (also known as *ABCB1*) and syndecan 4 (*SDC4*)[141], which in turn results in the expression of these genes being reinstated.

Importantly, use of these agents was adopted before the incidence of *DNMT3A* mutations was appreciated. Given the variable response to these agents, even among patients with similar disease phenotypes, a number of clinical studies were set up to examine the relationship between hypomethylating agent response and the mutational status of *DNMT3A* and other epigenetic modifiers. One study of 92 patients with MDS, MDS/MPN and sAML showed that patients with a *DNMT3A* mutation, a *TET2* mutation or both were more likely to have a favourable response to hypomethylating therapy[142]. Additionally, one small AML study found that decitabine treatment resulted in a higher complete remission rate and a trend towards increased overall survival in patients with *DNMT3A* mutations[123]. However, *in vitro* treatment with decitabine of primary samples from patients with AML did not lead to different responses in samples with *DNMT3A* mutations compared to those without[143]. Because *DNMT3A* mutations are likely to cause a reduction in DNA methyltransferase activity, the mechanism of therapeutic benefit provided by a drug that inhibits DNA methylation is puzzling. Studies including larger cohorts of patients are necessary to determine whether a true correlation exists between *DNMT3A* status and response to hypomethylating agents. Furthermore, there is poor correlation between changes in DNA methylation and gene expression after treatment with hypomethylating therapy, indicating that the mechanism of action of these agents is likely to be complex[143]. More work to delineate the mechanism of action of these drugs may improve our ability to predict which patients are most likely to benefit from treatment.

Given that *DNMT3A* mutations seem to have no clear impact on ability to achieve complete remission, the poor survival rate associated with such mutations is probably attributable to a high relapse rate[91,93,125,144]. Because *DNMT3A* mutations show remarkable stability during disease evolution, it is likely that the high relapse rate is due to the presence of *DNMT3A* mutations in ostensibly normal-appearing patient HSCs that persist even after chemotherapy and during relapse[82,83]. These mutant HSCs would be available to reinitiate disease after new oncogenic hits. With *DNMT3A*^{mut}-associated disease, long-term disease surveillance should be based on molecular, rather than histological, criteria.

The concept that *DNMT3A*-mutant progenitors are difficult to eradicate points to an urgent need to identify new approaches to specifically target these HSCs. The challenge will be the similarity of the mutant HSCs to their wild-type counterparts. In mice, *Dnmt3a*$^{-/-}$ HSCs retain most characteristics of normal stem cells, including relative quiescence[70,71]. One strategy may be to identify mechanisms to enforce mutant clone silence. For example, some patients with CML who are taken off tyrosine kinase inhibitor therapy after long treatment periods remain in remission despite the continued presence of breakpoint cluster region (*BCR*)–*ABL1*-positive cells, indicating that complete eradication of the leukaemic clone is not always necessary[145]. Alternatively, improved mechanistic understanding may lead to

new approaches, such as the upregulation of catalytically active *DNMT3B* splice isoforms. Considering the large number of patients with *DNMT3A* mutations across many haematological diseases, novel therapeutic approaches are greatly needed.

Conclusions

DNMT3A has recently emerged as one of the most important tumour suppressors in haematological malignancies. Its exceptional role is rooted in its crucial function in stem cells, in which it enables the first steps of haematopoietic differentiation. The observation that *Dnmt3a*$^{-/-}$ HSCs have a marked selective advantage over their normal counterparts in bone marrow underscores the Darwinian nature of competing HSCs in the haematopoietic system and suggests that certain mutations can have large effects on the stem cell pools over time. Indeed, the increasing prevalence of clonal haematopoiesis with age[146], which is highly associated with *DNMT3A* mutations[84–86], strongly suggests that stem cells that have acquired a *DNMT3A* mutation early in life may slowly accumulate, finally appearing in large numbers many years later.

In addition to age, we expect that other factors — including haematological stress, such as that caused by infection — could positively or negatively affect the proportion of normal versus mutant HSCs. Thus, the way that mutations in *DNMT3A* affect normal haematopoiesis, as well as the propensity of mutant clones to contribute to disease, will be of considerable future interest. These questions also have a crucial bearing on risk stratification and the choice of therapeutic modalities for patients.

On a mechanistic level, despite nearly two decades of basic research, we understand only poorly how DNMT3A carries out its duties with regard to DNA methylation and gene expression, and we have no inkling of any DNA methylation-independent functions.

In summary, DNMT3A has a crucial biological role in self-renewing cells, enabling their differentiation. When lost or reduced in activity, the balance is shifted, resulting in a predisposition to cancer and other pathological consequences. Further study of the different facets of this molecule, including basic research and more clinical data, should generate new insights, leading to new therapeutic opportunities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Imprinting

A genetic phenomenon in which the expression status of each allele of a gene is dictated by the parent from which the allele was inherited. DNA methylation plays a part in the crucial parent-specific regulation of gene expression.

CpG
A dinucleotide pair on the same DNA strand consisting of cytosine (C) and guanine (G) nucleotides joined by a phosphodiester bond (p); CpGs are the predominant target of DNA methylation.

Repetitive elements
Stretches of DNA that are found in multiple copies (often many thousands) throughout the genome. Most represent types of transposable elements, the activity of which is partly repressed by DNA methylation.

Pericentromeric heterochromatin
Regions of compact genomic DNA and chromatin that are located near centromeres. Pericentromeric heterochromatin is associated with repressive chromatin marks and inactive gene transcription.

Teratoma formation assay
A stringent test of pluripotency in which embryonic (or other) stem cells are transplanted into a mouse and examined for their ability to differentiate into all three germ layers.

Variant-allele frequencies (VAFs)
The relative proportions of sequencing reads from variant alleles. Variants found at a VAF of 50% usually represent heterozygous mutations that are present in all cells within the sample (the founding clone). Lower VAFs suggest that the variant occurs in only a fraction of the cells (possible subclone).

Compound heterozygous
The presence of two different mutant alleles at a particular gene locus, on each chromosome of a pair.

Myelodysplastic syndrome (MDS)
A group of myeloid disorders characterized by clonal and ineffective haematopoiesis, dysplasia in one or more of the myeloid cell lines, cytopaenia(s) and increased risk of the development of acute myeloid leukaemia.

Myeloproliferative neoplasms (MPNs)
A heterogeneous group of clonal haematopoietic stem cell disorders characterized by the abnormal proliferation of one or more of the myeloid lineages.

Chronic myelomonocytic leukaemia (CMML)
A clonal haematological malignancy with features of both a myeloproliferative neoplasm and a myelodysplastic syndrome. CMML is characterized by persistent monocytosis, dysplasia of one or more myeloid lineages, and <20% blasts in the blood and bone marrow. By definition, CMML lacks the breakpoint cluster region (*BCR*)–*ABL1* fusion gene and platelet-derived growth factor receptor-α (*PDGFRA*) or *PDGFRB* rearrangements.

Enhancers
Distal regulatory regions of the genome up to 1 megabase upstream of transcription start sites defined by chromatin marks such as monomethylation of histone H3 lysine 4 (H3K4me1) and acetylation of H3K27 (H3K27ac). Enhancers are often bound by transcription factors.

Secondary acute myeloid leukaemia (sAML)
A documented myelodysplastic syndrome or myeloproliferative neoplasm that transforms into AML. This subset of AML is now included in the category 'AML with myelodysplasia-related changes'.

Spliceosome factor
A member of the large and complex molecular machinery known as the spliceosome, which functions to remove introns from a transcribed precursor mRNA.

***De novo* acute myeloid leukaemia (*De novo* AML)**
Initial diagnosis of AML, not preceded by myelodysplastic syndrome or myeloproliferative neoplasm, and not associated with prior chemotherapy or radiation therapy.

Polycythaemia vera
A chronic myeloproliferative neoplasm characterized by aberrantly increased red blood cell production independent of the mechanisms that normally regulate erythropoiesis. Polycythaemia vera is molecularly characterized by activating mutations of the tyrosine kinase Janus kinase 2 (*JAK2*), which are present in nearly all patients with polycythaemia vera.

Essential thrombocythaemia
A chronic myeloproliferative neoplasm characterized by increased platelet count in the peripheral blood and megakaryocyte proliferation with large and mature morphology in the bone marrow. Essential thrombocytosis is characterized molecularly by activating mutations of the tyrosine kinase Janus kinase 2 (*JAK2*), which are present in 40–50% of patients with essential thrombocytosis.

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Box 1

DNMT3A, *TET2* and *IDH* mutations in lymphoma

In peripheral T cell lymphomas (PTCLs), combined aberration of DNA methylation and hydroxymethylation is likely to contribute to lymphomagenesis. However, unlike in myeloid disease, mutations in DNA methyltransferase 3A (*DNMT3A*) are highly correlated with concomitant mutations in the gene encoding a methylcytosine dioxygenase (*TET2*), with 70–100% of *DNMT3A*-mutant patients also harbouring mutations in *TET2* (REFS 101–103,147). In addition, in angioimmunoblastic T cell lymphoma (AITL; a PTCL subset), nearly two-thirds of patients with *TET2* mutations have two or three different *TET2* mutations, whereas such mutations are predominantly heterozygous in myeloid disease. Further, whereas in myeloid disease mutations of *TET2* and isocitrate dehydrogenase 1 (*IDH1*) and/or *IDH2* are mutually exclusive, they can co-occur in AITL, mostly in patients with only one *TET2* mutation[101]. These data strongly suggest that a substantial impairment of DNA hydroxymethylation — either from the loss of both normal copies of *TET2* or through a combination of *TET2* and *IDH2* mutation — is required for this disease to develop. Although the interaction between *DNMT3A* mutation and mutations in *TET2* and *IDH1* and/or *IDH2* in PTCL is distinct from that in myeloid malignancies, importantly PTCL has a significantly different pattern of mutations when compared with T cell acute lymphoblastic leukaemia (T-ALL). In both T-ALL and PTCL, non-R882 *DNMT3A* mutations predominate; however, biallelic *DNMT3A* mutations are rarely encountered in PTCL, whereas most patients with T-ALL have them. There are at least two possible explanations for these interesting findings: first, with such profound loss of DNA hydroxymethylation in PTCL, less impact on DNA methylation is necessary for disease development; or second, retention of at least partial DNMT3A function is necessary for these diseases to develop.

Box 2

DNMT3A and alternative splicing

Epigenetic regulation through histone modifications has been linked to alternative splicing[148,149], perhaps also implicating DNA methylation. Gene body DNA methylation is associated with transcriptionally active genes[150], the exons of which are preferentially marked by histone H3 lysine 36 trimethylation (H3K36me3)[151]. DNA methyltransferase 3A (DNMT3A) physically interacts with H3K36me3 (REF. 28), linking DNMT3A to exon methylation. Interestingly, a spike of DNA methylation is observed at the 5′ splice site or exon–intron junction, followed by a sharp plummet at the 3′ splice site[152]. Moreover, in maize, high levels of DNA methylation at the 5′ spice site correlate with higher rates of alternative splicing[153], linking DNA methylation to alternative splicing. With these observations, we can speculate that DNMT3A might have a role at the 5′ splice site. Intriguingly, a number of spliceosome factors that are co-mutated with *DNMT3A* in myelodysplastic syndrome are assigned specifically to the 3′ splice junction[154]. The implications of DNA methylation for splicing, and its role in diseases in which mutations in spliceosome factors are found, warrant further exploration.

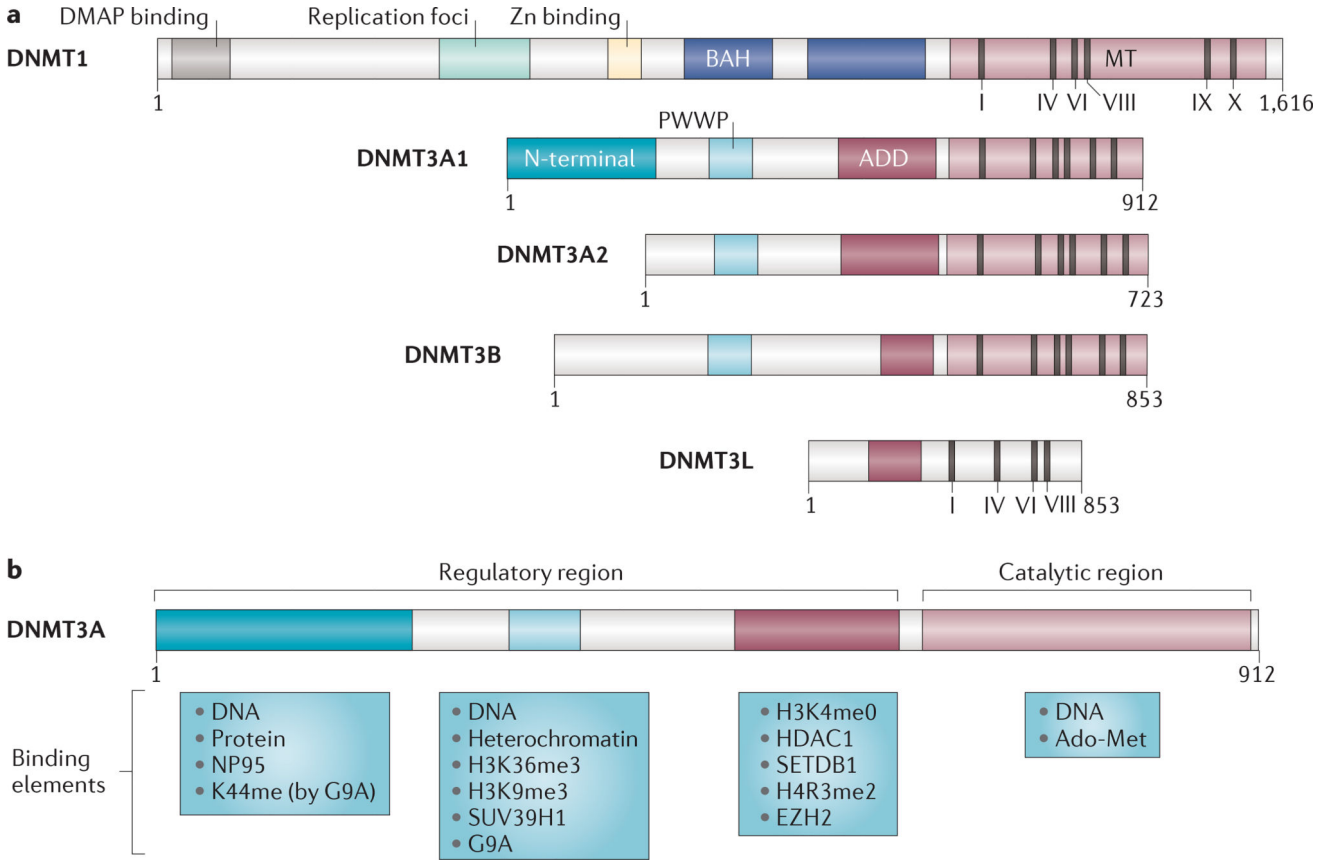


Figure 1. The structure of DNA methyltransferase proteins and their binding partners

a | Domain architecture of DNA methyltransferase 1 (DNMT1), DNMT3A1, DNMT3A2, DNMT3B and DNMT3-like (DNMT3L) and major DNMT3A splice isoforms. Note that DNMT3B has a number of additional isoforms that are not depicted here[36,37]. Protein length is indicated (length given as number of amino acids). Domain abbreviations: ADD, ATRX-DNMT3-DNMT3L (related to the plant homology (PHD)-like domain of regulator ATRX); BAH, Bromo adjacent homology domain; DMAP, DNMT1-associated protein; PWWP, Pro-Trp-Trp-Pro. MT is the catalytic methyltransferase domain, and I, IV, VI, IX and X are motifs in the catalytic domain: motif I allows the binding of the methyl group donor AdoMet (*S*-adenosyl methionine). Motifs I and X are for cofactor binding, and motifs VIII and IX are for DNA binding. The catalysis of DNA methylation occurs at the IV, VI and VIII motifs[155].

b | DNMT3A contains an amino-terminal domain that is unique to the long isoform and exhibits DNA-binding capability[27,156]. It may also interact with transcription factors such as OCT3 (also known as OCT4 and POU5F1) in embryonic stem cells[157]. Lysine 44 (K44) of the N-terminal domain is dimethylated by G9A and is important for interactions with G9A and/or EHMT1 (also know as GLP)[50,51]; this interaction is necessary for the DNA methylation of some loci, such as the OCT3 promoter, which is involved in embryonic stem cell pluripotency. PWWP has diverse capabilities, including DNA[156] and heterochromatin binding[40,158], as well as interactions with the histone H3 lysine 36 trimethylation (H3K36me3) and H3K9me3 marks[28]. The ADD domain has strong affinity for histone deacetylase 1 (HDAC1)[29] and for unmodified H3K4 (REFS 30–32), and is thought to

positively affect DNA methyltransferase activity[159].This domain binds to the methyltransferase domain to act as an auto-inhibition loop in the presence of unmodified histone H3 binding[32]; it also interacts with histone modifiers involved in gene repression, such as the histone-lysine *N*-methyltransferase SUV39H1 (REF. 53). Furthermore, the ADD domain binds to the histone-lysine *N*-methyltransferase enhancer of zeste homologue 2 (EZH2); EZH2 is part of Polycomb repressive complex 2 (PRC), which is responsible for H3K27 methylation[52,53].

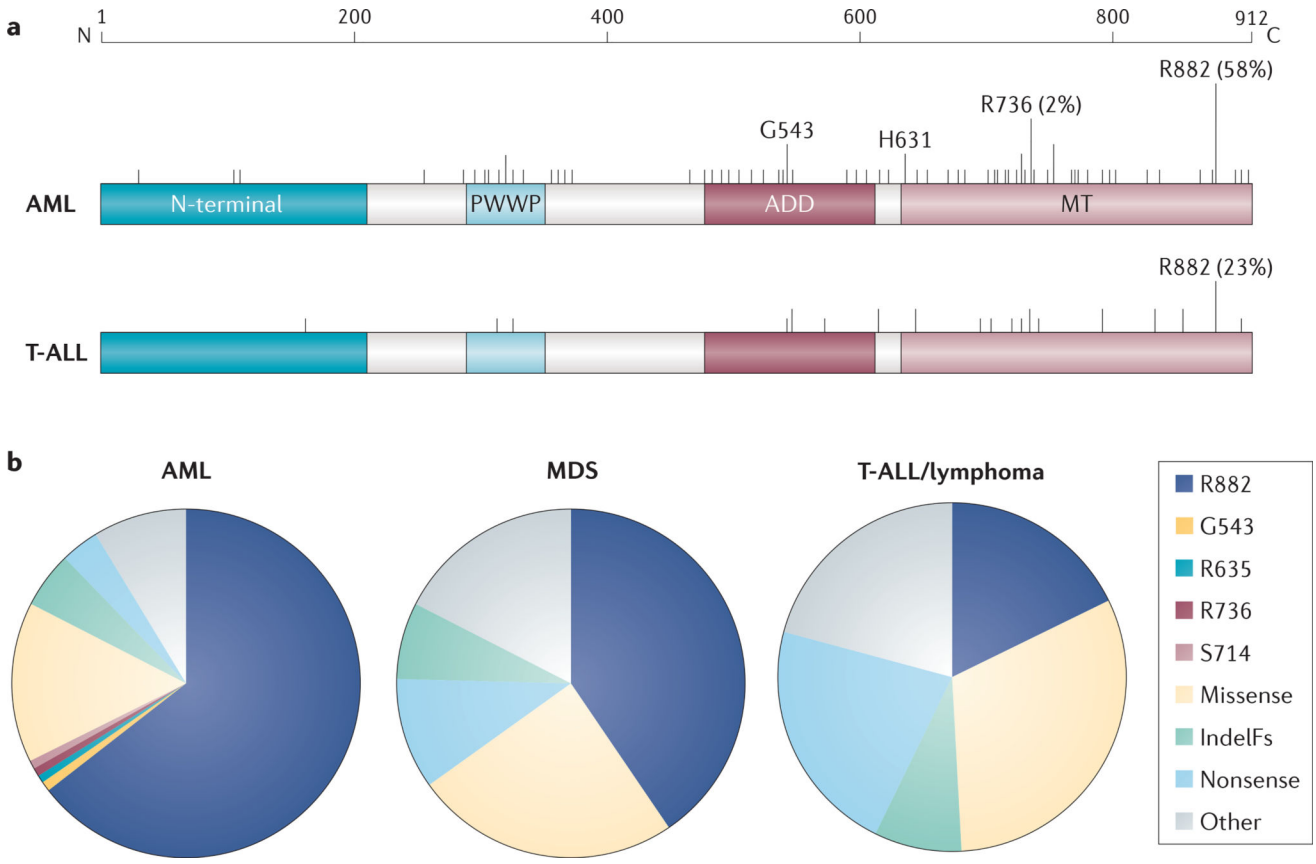


Figure 2. Distribution of DNMT3A mutation frequency in myeloid and lymphoid leukaemia

a | Structural diagram of the location of mutations in DNA methyltransferase 3A (DNMT3A). The top band indicates amino acid position and scale. The vertical lines show the mutations mapped to DNMT3A in acute myeloid leukaemia (AML) and T cell acute lymphoblastic leukaemia (T-ALL) in the subset of patients in which the entire gene has been sequenced, as curated from the published literature. Long lines represent >0.03% frequency of mutation. Also shown is the distinct frequency of mutation at arginine 882 (R882) in AML versus T-ALL, and the minor hotspot in AML G543 (1.4%) and R736 (2.1%) residues. **b** | Frequency of mutations in AML, myelodysplastic syndrome (MDS) and T-ALL/lymphoma, as curated from the Catalogue of Somatic Mutations in Cancer (COSMIC) database (see Further information). ADD, ATRX-DNMT3-DNMT3L; IndelFs, insertion–deletion frameshift mutations; MT, methyltransferase; PWWP, Pro-Trp-Trp-Pro.

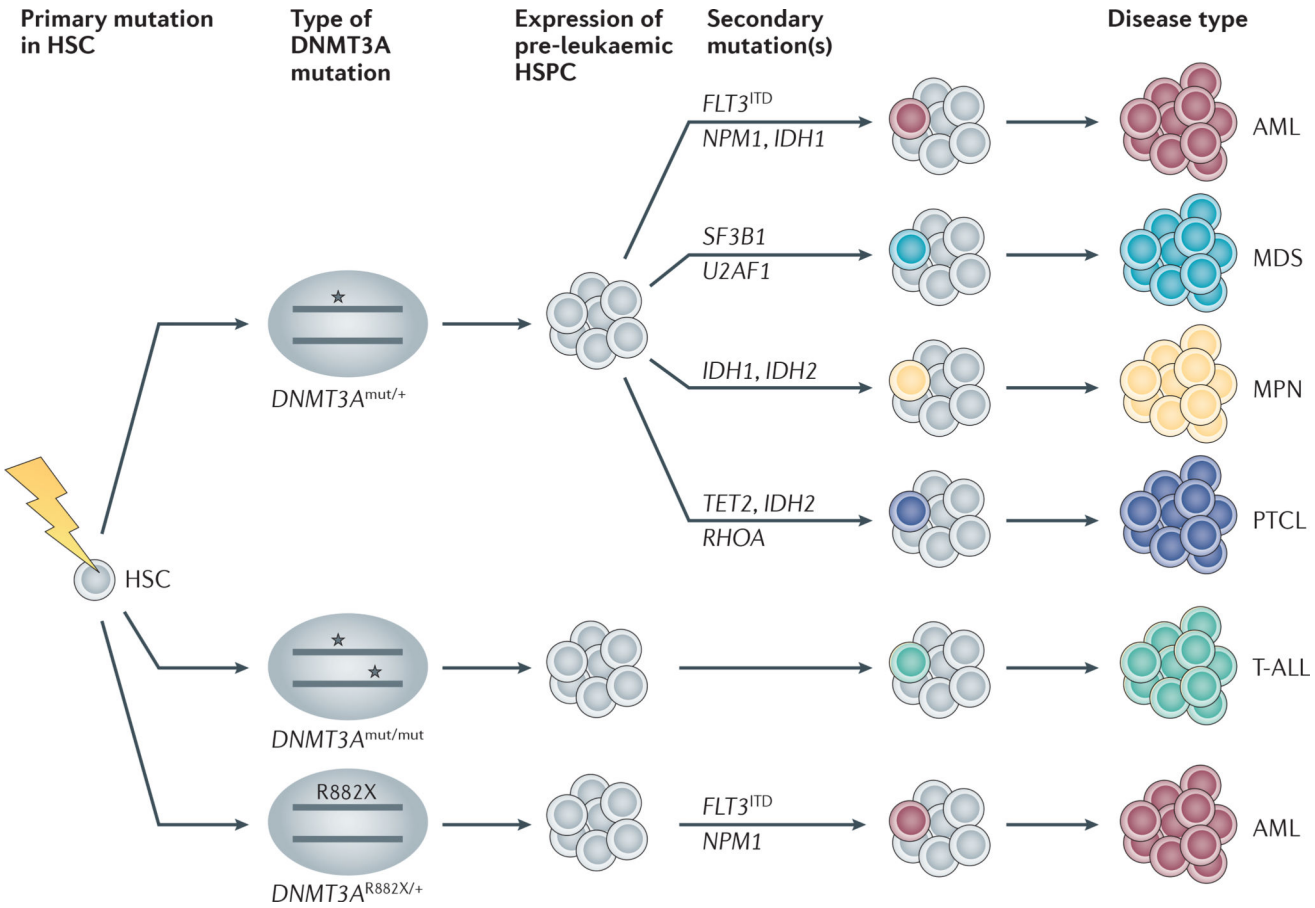
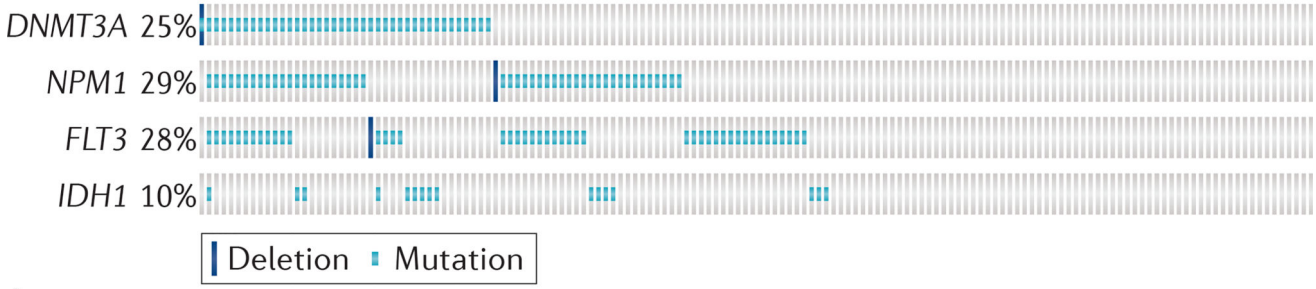


Figure 3. DNMT3A mutation allele and gene dosage, combined with secondary mutations, are likely to dictate the type of haematological disease

DNA methyltransferase 3A (*DNMT3A*) mutations (indicated by stars) are likely to arise in the pre-leukaemic haematopoietic stem cell (HSC) compartment, in which heterozygous mutations predispose the occurrence of myeloid disease and peripheral T cell lymphoma (PTCL), whereas homozygous mutations are likely to occur in T cell disease. Certain mutations in R882X, where X is an amino acid other than R, lead to the acquisition of co-mutations; that is, internal tandem duplication in the gene encoding the receptor tyrosine kinase FLT3 (*FLT3*^{ITD}) and mutations in the gene encoding nucleophosmin (*NPM1*). Acquisition of a secondary mutation in myeloid disease is associated with distinct myeloid neoplasms, including acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPNs). HSPC, haematopoietic stem and progenitor cell; *IDH*, isocitrate dehydrogenase; mut, mutant; *RHOA*, RAS homologue family member A; *SF3B1*, splicing factor 3b, subunit 1; T-ALL, T cell acute lymphoblastic leukaemia; *U2AF1*, U2 small nuclear RNA auxiliary factor 1.

a TCGA-AML



b

Disease	Positive correlation	No association	Negative correlation
AML	• Normal karyotype • *NPM1* • *FLT3*^{ITD} • *IDH1* • Cohesin complex*	• *TET2* • *IDH2* • *ASXL1* • Spliceosome complex mutations	• t(8;21) • inv(16) • *KMT2A* rearrangements
MDS	• *SF3B1* • *U2AF1*	• *TET2* • *IDH1* and *IDH2*‡	• *SRSF2* • *ASXL1*
MPN	*IDH1* and *IDH2*	*JAK2*	–
PTCL	• *TET2* • *IDH2* (*AITL*) • *RHOA*	–	–
T-ALL	Normal karyotype	• *FLT3*^{ITD} • *IDH1* and *IDH2* • *NOTCH1* • *PHF6*	*CDKN2A* and *CDKN2B* deletion

Figure 4. Co-mutations with DNMT3A in acute myeloid leukaemia and other diseases

a | Mutations in nucleophosmin (*NPM1*) and internal tandem duplication in the gene encoding the receptor tyrosine kinase FLT3 (*FLT3*^{ITD}) occur more frequently in DNA methyltransferase 3A (*DNMT3A*)-mutant acute myeloid leukaemia (AML) than in non-*DNMT3A*-mutant AML. Each column represents a patient from The Cancer Genome Atlas (TCGA) database. Each coloured mark represents a mutation (light blue) or deletion (dark blue). The frequency of patients with each mutation is indicated on the left-hand side. The figure was made using cBioPortal[160,161]. **b** | Frequent co-mutations with *DNMT3A*. AITL, angioimmunoblastic T cell lymphoma; *ASXL1*, additional sex combs-like transcriptional regulator 1; *CDKN*, cyclin-dependent kinase inhibitor; *IDH*, isocitratedehydrogenase; *JAK2*, Janus kinase 2; *KMT2A*, histone-lysine *N*-methyltransferase KMT2A (also known as *MLL*); MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; *PHF6*, PHD finger protein 6; PTCL, peripheral T cell lymphoma; *RHOA*, RAS homologue family member A; *SF3B1*, splicing factor 3b, subunit 1; *SRSF2*, serine/arginine-rich splicing factor 2; T-ALL, T cell acute lymphoblastic leukaemia; *U2AF1*, U2 small nuclear RNA auxiliary factor 1.*One large study showed significant association, but another study found no association.

‡One study found a significant association with *IDH2* mutations that was not confirmed in two additional large studies.

Table 1

Frequency of DNMT3A mutations in haematological disorders

Disease	Patient population	Frequency (%)	Refs
AML	*De novo* AML	62/281 (22.1)	76
	CN AML	44/120 (36.7)	
	De novo AML	70/500 (14)	147
	CN AML	51/223 (22.9)	
	<60 years CN AML	64/181 (35.5)	123
	≥60 years CN AML	77/234 (33.3)	
	sAML	13/37 (35) (10/27 (37) from MDS; 3/10 (33) from MPN)	126
	tAML	10/59 (17)	
	Paediatric AML	3/140 (2.1)	115
MDS	Adult *de novo* MDS	127/944 (13.5)	121
	Paediatric MDS, tMDS	0/44 *	117
MPN	MPN (entire cohort)	10/155 (9)	98
	PMF	1/16 (6)	
	PV	2/30 (7)	
	ET	0/30 (0)	
	Blast phase MPN	5/35 (14)	
	Systemic mastocytosis	3/26 (12)	162
	CML	0/79	163
CMML and JMML	CMML	5/227 (2) *	164
	CMML-1	1/48 (2)	100,134
	CMML-2	1/16 (6.3)	
	sAML from CMML	6/23 (26)	
	JMML	1/113 (0.8)	165
T cell leukaemia and lymphoma	T cell lymphoma	11/96 (11)	147
	PTCL	21/79 (26.6)	103
	PTCL-NOS	9/33 (27.3)	
	AITL	12/46 (26.1)	
	Adult T-ALL (entire cohort)	16/83 (18)	104
	Early (pro- and pre-leukaemic) T cell leukaemia	10/38 (26.3)	
	Cortical T-ALL	5/39 (12.8)	
	Adult ETP ALL	11/68 (16)	137
	Paediatric T-ALL	0/91	79
	Paediatric ETP ALL	0/12	119
MPAL	Adult T cell and myeloid MPAL	10/18 (55.6)	166
	Paediatric mixed lineage leukaemia	0/20	79

The range of frequencies reported within a given disease type depends on the subset of patients examined as well as whether the entire *DNMT3A* gene or just the hotspots were examined. Also see the comprehensive online Supplementary information S1 (table). AITL, angioimmunoblastic T cell lymphoma; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; CN AML, cytogenetically normal AML; ET, essential thrombocytopaenia; ETP, early T cell precursor; JMML, juvenile myelomonocytic leukaemia; MDS, myelodysplastic syndrome; MPAL, mixed phenotype acute leukaemia; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PTCL, peripheral T cell lymphoma; PTCL-NOS, PTCL-not otherwise specified; PV, polycythaemia vera; sAML, secondary AML; T-ALL, T cell ALL; tAML, therapy-related AML (AML that develops after exposure to cytotoxic chemotherapy and/or radiation therapy administered for a prior neoplastic or non-neoplastic disorder); tMDS, therapy-related MDS.

* Only sequenced exon 23.

Table 2

Biochemical impact of mutations in DNMT3A found in cancer patients

Residue (murine)	AA	Disease	Domain	Consequence	Refs
308 (304)	Q	AML	PWWP	H3	159
664 (660)	E	AML	Motif II	Cat	60
710 (706)	C	sAML from MDS	Motif IV	Cat, DNA, AdoMet (−)	60
714 (710)	C	AML	Following motif IV	AdoMet	60
720 (716)	N	T-ALL	Following motif IV	Cat, DNA	60
729 (725)	R	AML	Catalytic	DNA	106
733 (729)	E	AML, CMML	Catalytic	DNA	106
771 (767)	R	AML, MDS, SM	Catalytic	DNA	106
792 (788)	R	T-ALL	Motif VIII	Cat, DNA (+)	60
826 (822)	K	MDS	Catalytic	DNAMulti	167
841 (837)	K	AML	Catalytic	DNAMulti	167
856 (852)	E	T-ALL	Catalytic	DNA (+)	167
860 (856)	W	AML	Catalytic	Cat	105
879 (875)	N	AML	Catalytic	Cat	105
882 (878)	R	AML	In front of motif X	Cat, DNA	60

AA, amino acid; AdoMet, *S*-adenosyl methionine reduced AdoMet binding; AdoMet (−), no AdoMet binding; AML, acute myeloid leukaemia; Cat, reduced catalytic activity; CMML, chronic myelomonocytic leukaemia; DNA, reduced DNA binding; DNA (+), enhanced DNA binding; DNAMulti, loss of ability to multimerize on DNA; DNMT3A, DNA methyltransferase 3A; H3, insensitive to H3 peptide; MDS, myelodysplastic syndrome; PWWP, Pro-Trp-Trp-Pro; sAML, secondary AML; SM, systemic mastocytosis; T-ALL, T cell acute lymphoblastic leukaemia.