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Review

Role of somatic cancer mutations in human protein lysine methyltransferases

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ABSTRACT

Methylation of lysine residues is an important post-translational modification of histone and non-histone proteins, which is introduced by protein lysine methyltransferases (PKMTs). An increasing number of reports demonstrate that aberrant lysine methylation plays a central role in carcinogenesis that is often correlated with abnormal expression of PKMTs. Recent whole genome and whole transcriptome sequencing projects have also discovered several somatic mutations in PKMTs that frequently appear in various tumors. These include chromosomal translocations that lead to aberrant expression or mistargeting of PKMTs and nonsense or frameshift mutations, which cause the loss of the protein function. Another type of mutations are missense mutations which may lead to the loss of enzyme activity, but may also alter the properties of PKMTs either by changing the product or substrate specificity or by increasing the enzymatic activity finally leading to a gain-of-function phenotype. In this review, we provide an overview of the roles of EZH2, SETD2, NSD family, SMYD family, MLL family and DOT1L PKMTs in cancer focusing on the effects of somatic cancer mutations in these enzymes. Investigation of the effect of somatic cancer mutations in PKMTs is pivotal to understand the general role of this important class of enzymes in carcinogenesis and to improve and develop more individualized cancer therapies.

Contents

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1. Introduction

1.1. Role of epigenetic modifications in chromatin biology

All human cells carry the same genetic information, yet they follow different developmental pathways and differentiate into the more than 100 different cells types found in the human body. The cellular fate and phenotype is determined by epigenetic regulatory mechanisms, including DNA methylation, modifications of the histone tails and non-coding RNAs, which all cooperate to modulate chromatin structure and, thereby, control gene expression [1–7]. The basic functional unit of chromatin is the nucleosome, which contains 147 base pairs of DNA wrapped around a core histone octamer comprising of two subunits of each of the histones H2A, H2B, H3, and H4. Fundamentally, chromatin is subdivided into two major parts: i) heterochromatin, which is highly condensed, late to replicate, and primarily contains inactive genes; and ii) euchromatin, which is more open and contains most of the active genes. Modifications of the DNA and histones are dynamically set and removed by chromatin-modifying enzymes in a highly regulated manner. These modifications can alter the chromatin structure by modulation of non-covalent interactions within and between nucleosomes. They also serve as docking sites for specialized proteins with unique domains that specifically recognize these modifications. These chromatin readers recruit additional chromatin modifiers and remodeling enzymes, which serve as the effectors of the modification.

Epigenetic signals include DNA methylation, non-coding RNAs and modifications of the histone proteins mainly within the N-terminal tails of H3 and H4, which are subject to a variety of post-translational modifications like acetylation, methylation, phosphorylation or ubiquitylation. Methylation of histone tails can occur on Lys and Arg residues [7–10]. Lysine methylation is introduced by a class of enzyme called lysine methyltransferases (PKMTs) [11,12], most of which contain a SET domain as catalytic unit, named after the *Drosophila* proteins Su(var)3-9, Enhancer-of-zeste and Trithorax in which it was initially characterized. The catalytic SET domain of PKMTs is often fused to additional regulatory and targeting domains.

Lysine methylation of histone proteins can have diverse effects depending on the modified residue. For example, trimethylation of H3K4 is generally associated with active transcription while trimethylation of H3K9, H3K27 and H4K20 is associated with repressed chromatin. In addition, the different methylated forms of the lysine side chain (K^{me1}, K^{me2} and K^{me3}) add another layer of complexity and each form of lysine methylation can have a specific biological effect. In the case of H3K4 methylation, for example, H3K4me1 is found at gene enhancers while H3K4me3 is associated with the transcriptional start site of active genes. To give another example, the different methylated forms of H3K27 also have different functions; H3K27 mono-methylation is found in the gene bodies and positively correlates with the expression of less active genes but negatively correlates with the highly expressed genes [13]. In contrast, H3K27 trimethylation is usually found in the promoter regions of developmental genes and is a hallmark of gene repression [14].

The methylation of Lys can be removed by lysine demethylase enzymes (KDMs) belonging to either the LSD family or enzymes containing JumonjiC (JmjC) domains [15]. LSD1 and LSD2 are FAD dependent amine oxidases that catalyze the removal of a single methyl group from mono and dimethylated lysine residues. The second and much larger family of JmjC domain containing KDMs comprises about 20 human enzymes that are further grouped into 5 subfamilies. These enzymes utilize α-ketoglutarate and Fe(II) ions as cofactors in the hydroxylation of lysine bound methyl groups. They catalyze the removal of mono-, di- and trimethylated lysines at various sites of the histone tails. Similar to PKMTs, aberrant regulation and expression of KDMs are also involved in cancer progression [16,17], but this will not be further discussed in this review.

1.2. Examples of somatic cancer mutations in epigenetic enzymes

Cancers arise as a result of the acquisition of a series of mutations and epigenetic changes, many of which ultimately confer a growth advantage upon the cells in which they have occurred [18–21]. In recent years, the rapid development of novel DNA sequencing techniques has revolutionized biomedical research. Among other important advances, various genome-wide exome sequencing studies revealed several genes that are frequently somatically mutated in tumor tissues [22]. The Cosmic "Catalogue Of Somatic Mutations In Cancer" database at http://www.sanger.ac.uk/genetics/CGP/cosmic/ provides a useful compilation of these data [23]. Histone methylation and other epigenetic modifications were known to have important roles in disease including cancer [7,19–21,24,25]. The systematic sequencing projects revealed that many of the genes containing somatic cancer mutations have epigenetic roles, either directly or indirectly. This prevalence of somatic mutations in chromatin-modifying genes highlights the central role of transcriptional dysregulation in tumorigenesis.

Like in other genes, somatic mutations of epigenetic factors can lead to either loss-of-function or gain-of-function. Loss-of-function mutations include critical point mutations, frame shifts, nonsense mutations and deletions that cause an inactivation of the affected protein. In contrast, gain-of-function mutations lead to a change in the function of the affected protein, which may contribute to the cancerogenic process. In case of such gain-of-function mutations, often the alteration of one allele is sufficient, whereas loss-of-function mutations typically affect both alleles (except in cases of haploinsufficiency). While loss-of-function mutations often can be understood on the basis of the known functions of the gene product, gain-of-function mutations hold a particular challenge, because their understanding requires an in depth experimental investigation of the affected protein and each specific mutation, which makes their understanding difficult. On the other hand, they provide unique information about cancerogenic pathways that may aid future therapeutic development. In addition, they hold the promise that a specific inhibition of a mutant version of a cancer gene could serve as a specific therapeutic approach in an individualized therapy.

Examples of frequently mutated epigenetic enzymes include the DNA methyltransferase 3a (DNMT3a), the ten–eleven-translocation 2 methylcytosine hydroxymethyltransferase (TET2), isocitrate dehydrogenase (IDH) 1 and 2 and SWI/SNF chromatin remodeling complexes. DNMT3a is an essential DNA methyltransferase [5,6]. The very frequently observed R882H mutation in Dnmt3a reduces its activity and changes its oligomerization [26,27] and by this it may alter the DNA methylation pattern. Somatic TET2 mutations are frequently observed in various cancers [28], and the mutations include nonsense, deletion and missense mutations at very crucial residues for the enzymatic activity thus predicted to inactivate the enzyme [29]. This mutational pattern strongly suggests that mutations act by inactivating the enzyme and TET2 acts as a tumor suppressor [30]. In addition, many somatic cancer mutations were detected in IDH, which unexpectedly turned out to act

as indirect epigenetic gain-of-function mutations. IDH is a key enzyme in the Krebs cycle, which catalyzes the conversion of isocitrate to α-ketoglutarate (αKG). There exist two isoforms of IDH, IDH1 in the cytosol and IDH2 in the mitochondria. All discovered IDH mutations in various cancers reside in the active site of the enzyme and they occur heterozygously. It has been shown that the mutant forms of IDH cannot catalyze the conversion of isocitrate to αKG, but they catalyze its NADPH-dependent reduction 2-hydroxyglutarate (2-HG) [31,32]. 2-HG is normally only present at very low levels in cells, but it is elevated in IDH-mutant cells. It has a similar structure as αKG and acts as a competitive inhibitor for αKG dependent dioxygenases including TET enzymes [33,34] and the Jumonji family of histone lysine demethylases [35]. In agreement with this observation, hypermethylation of many histone methylation marks was observed following an introduction of IDH1/2 mutations into cells [35] and DNA hypermethylation has been shown as well [36]. Finally, mutations that inactivate SWI/SNF chromatin remodeling complexes subunits are found in nearly 20% of human cancers [37]. These complexes catalyze the opening or condensation of chromatin and by this help to execute the epigenetic program.

1.3. Somatic cancer mutations in PKMTs

This review will focus on the discussion of somatic cancer mutations in PKMTs (Table 1). The analysis of the available data taken from COSMIC allows identifying candidate PKMTs, which show enrichment of cancer mutations (Fig. 1A). The strongest enrichment is observed in EZH2, but MLL2, MLL3, SET7/9, SETD2, SYMD1, SMYD3, and SUV420H1 also show relatively high frequencies of somatic cancer mutations when compared to the size of the proteins. Of note, this list may be highly influenced by sample bias, because the initial observation of mutations in one enzyme will always stimulate follow-up work that may lead to the discovery of additional mutations. In addition, the mutational spectrum depends on the tumor type, but currently the database is dominated by blood cancers (due to the relative ease of preparing pure tumor cells for analysis). Therefore, the picture may change in future when more data for various other cancers will become available. Using the COSMIC database, the position of the mutations in the protein and its domains can also be displayed as well as the cancer type distribution of mutations (see Fig. 2A–C for an example using the SUV39H2 enzyme).

1.4. Possible roles of somatic cancer mutations in PKMTs

In this review, we will focus on somatic cancer mutations in PKMTs most of which have roles in histone methylation. These enzymes often are also called histone methyltransferases, although several reports showed that many of them also methylate non-histone proteins. For example, we reported that G9a and SET7/9 methylate several non-histone substrates [38,39]. Various other non-histone substrates of PKMTs were compiled recently in excellent reviews [40–42] and there is consent in the field that many more human proteins are substrates for PKMTs. In the following chapters, we first will summarize the known roles of different PKMTs in cancer and then describe the occurrence and potential roles of somatic mutations in these enzymes.

As in other epigenetic enzymes, somatic cancer mutations in PKMTs could have loss-of-function or gain-of-function effects. While frameshifts and deletion mutations will mainly have loss-of-function effect, the situation is less clear for missense mutations, which cause an amino acid exchange in the protein. Such point mutations in PKMTs could inactivate the enzyme, if they disrupt AdoMet binding, folding or the binding of the substrate peptide. However, point mutations can also have gain-of-function effects, like the alteration of the peptide interaction potentially leading to the methylation of novel substrates, the change of the product methylation state, or an alteration of the

Table 1
Compilation of somatic cancer mutations in PKMT full length proteins listed in the COSMIC database (retrieved in May 2014). Synonymous mutations were not included. The Mutational score reports the ratio of the total number of mutations divided by the length of the corresponding protein in amino acids. The Sense score reports the ratio of the missense mutations (which depending on the exchange will cause loss of function or gain of function effects) dived by the sum of the number of frameshift and nonsense mutations (which will usually cause loss of function effects). For SUV39H1 the Sense score is not defined, since the denominator is zero.

PKMT	No. of somatic cancer mutations					Protein length (aa)	Mutational score	Sense score
	Frame shift	Nonsense	Missense	Other	Total			
DOT1L	1	9	72	6	88	1739	0.05	7.2
ESET (SETDB1)	4	6	71	5	86	1291	0.07	7.1
EZH1	1	7	48	1	57	747	0.08	6.0
EZH2	37	28	473	41	579	751	0.77	7.3
G9a	1	3	40	0	44	1210	0.04	10
GLP (EuHMT1)	0	1	75	3	79	1298	0.06	75
MLL1 (KMT2A)	26	27	190	5	248	3969	0.06	3.6
MLL2 (KMT2D)	82	112	294	26	514	5537	0.09	1.5
MLL3 (KMT2C)	82	117	436	24	659	4911	0.13	2.2
MLL4 (KMT2B)	19	22	133	7	181	2715	0.07	3.2
MLL5	17	9	102	6	134	1858	0.07	3.9
NSD1	10	10	130	6	156	2696	0.06	6.5
NSD2	4	4	84	2	94	1365	0.07	11
NSD3	1	5	33	0	39	1437	0.03	5.5
SET1A	10	6	104	0	120	1708	0.07	6.5
SET7/9 (SETD7)	1	1	31	2	35	366	0.10	16
SETD1A	10	6	104	0	120	1707	0.07	6.5
SETD1B	6	1	21	0	28	1923	0.01	3.0
SETD2	46	77	198	18	339	2062	0.16	1.6
SETD8 (PR-SET7)	0	1	19	3	23	352	0.07	19
SETDB2	3	3	34	0	40	719	0.06	5.7
SMYD1	3	3	60	1	67	490	0.14	10
SMYD2	2	2	24	1	29	433	0.07	6.0
SMYD3	2	3	24	5	34	369	0.09	4.8
SMYD4	1	0	40	1	42	804	0.05	40
SMYD5	1	2	19	1	23	418	0.06	6.3
SUV39H1	0	0	23	0	23	412	0.06	n.d.
SUV39H2	0	2	29	0	31	410	0.08	15
SUV420H1	5	6	75	1	87	885	0.10	6.8
SUV420H2	1	0	14	0	15	462	0.03	14

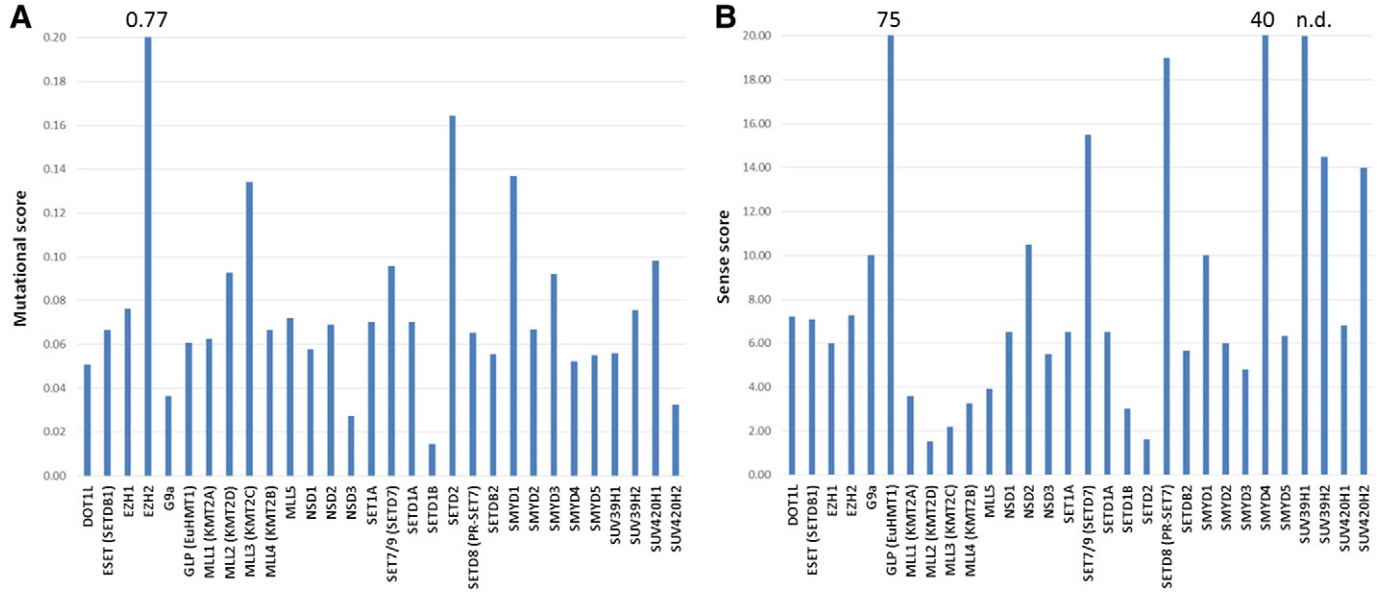

Fig. 1. Graphical representation of the Mutational score (A) and Sense score (B) of PKMTs. Data were taken from Table 1. The Mutational score reports the ratio of the total number of mutations divided by the length of the corresponding protein in amino acids. The Sense score reports the ratio of the missense mutations (which depending on the exchange will cause loss of function or gain of function effects) divided by the number of frameshift and nonsense mutations (which will usually cause loss of function effects).

interaction with other proteins leading to changes in the regulation or targeting of the PKMT (Fig. 3). Depending on the original role of the mutated PKMT in the regulation of a cancer related genes, somatic mutations in PKMTs can lead to the overexpression of oncogenes or loss of expression of tumor suppressor genes via different pathways (Fig. 4).

To allow an initial prediction on the main effect of somatic cancer mutations in each PKMT, we have calculated the "Sense score" of the mutational spectrum of each PKMT (Fig. 1B), which we define as the ratio of the missense mutations divided by the sum of frameshift and nonsense mutations. This number corresponds to the probability of a widespread gain-of-function mechanism, because a missense mutation has the potential to cause gain-of-function effects, while frameshift or nonsense mutations usually will lead to loss-of-function. Thus, a high Sense score as seen in SUV39H1, GLP, SET7/9, SUV420H2 or SETD8 proposes a gain-of-function effect of many mutations in these enzymes, while low Sense scores as in MLL2, MLL3 or SETD2 predicts a loss-of-function mechanism in many cases. However, this score can only serve as a rough approximation, since each mutation obviously has its own effect on the enzyme that may differ from the "average" effect of all other mutations. In the following chapters, we will introduce several important families of PKMTs, briefly summarize their role in cancer and describe the effects of somatic cancer mutations in the respective enzymes. We will also include information about changes of the expressional level of PKMTs although this is not the main focus of this work. These data can be retrieved from the Oncomine database (http://www.oncomine.org/), as shown in Fig. 2D using SUV39H2 for illustration.

2. Roles of PKMTs and their known somatic mutations in cancer

2.1. The EZH2 H3K27 PKMT

The Polycomb repressive complex 2 (PRC2) introduces H3K27me3, which is an important repressive chromatin mark. Polycomb proteins were initially identified in *Drosophila melanogaster* as developmental regulators [43]. In mammals, the PRC2 core complex consists of EED, SUZ12, NURF55, Rbap46/48 and the catalytic subunits EZH1 or EZH2. It also has some variable additional subunits including PHF1, JARID2 and AEBP2, which further regulate the PRC2 activity [44]. The SET domain containing EZH1 and EZH2 PKMTs can introduce up to three methyl groups on H3K27 [45]. H3K27 methylation is majorly achieved by the PRC2-EZH2 complex and only to a lesser extent by PRC2-EZH1 complexes, nevertheless, PRC2-EZH1 is able to efficiently repress transcription and compact the chromatin independent of its methyltransferase activity [46]. Polycomb proteins play an important role in epigenetic gene silencing in X-chromosome inactivation and imprinting. In addition, Polycomb targets include transcription factors, signaling genes that play a main role in cell fate determination and tumor suppressor genes, which function to prevent uncontrolled cell proliferation. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking these two epigenetic repression systems.

2.1.1. The role of EZH2 in cancer

Mutations in Polycomb proteins cause developmental defects due to the deregulation of gene expression of key transcription factors, such as the Homeobox proteins [47]. Polycomb-mediated silencing of those genes prevents the cell from undergoing senescence, which might ultimately lead to the onset of cancer. EZH2 overexpression is observed in tumors and it correlates with a poor prognosis in several tumor types [48]. EZH2 overexpression can lead to the hyper-silencing of differentiating genes or tumor suppressor genes, which can be reverted pharmacologically [47]. Later studies reported that EZH2 has both oncogenic and tumor suppressive roles [49]. EZH2 has been described also to methylate non-histone proteins including K299 of the transcription factor GATA4 [50] or K38 of retinoic acid receptor alpha (RORα) [51], which also could have an effect in carcinogenesis.

2.1.2. Somatic cancer mutations in EZH2

Next-generation sequencing of follicular lymphoma and diffuse-large B-cell lymphoma has revealed frequent somatic, heterozygous mutations at Y641 and A677 within the catalytic SET domain of EZH2 [52–54]. Heterozygosity and the presence of equal quantities of both mutant and normal mRNA and expressed protein suggest a dominant gain-of-function mode of action. The Y641 residue was the most frequently mutated residue, with up to 22% of cancer cells harboring mutations at this site. B-cell lymphoma cell lines and lymphoma samples harboring heterozygous EZH2 Y641 mutations have increased levels of histone H3K27me3. This residue is part of the so-called aromatic cage in the active center of EZH2 that surround the target lysine ε-aminogroup. Alterations of aromatic cage residues in PKMTs have been shown to affect

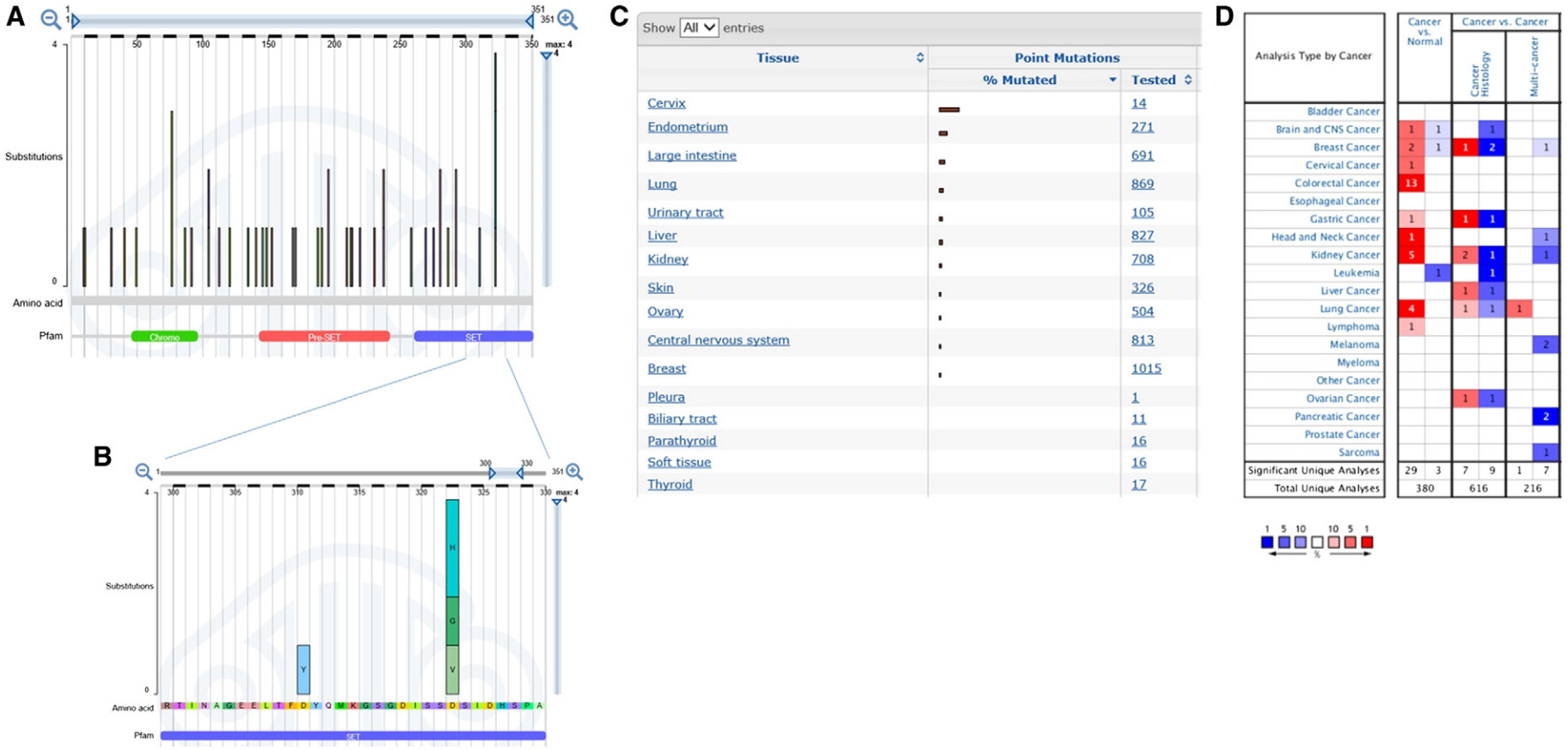

Fig. 2. Examples of the visualization of the distribution of somatic cancer mutations and expression changes of PKMTs using public databases and Web servers. A) Distribution of mutations in the SUV39H2 PKMT and domain structure of SUV39H2 showing the location of its Chromo, Pre-SET and SET domains. B) Zoom-in highlighting the mutations found at amino acid residue D322. C) Cancer type distribution of somatic cancer mutations in SUV39H2. D) Expression changes of SUV39H2 in cancer tissues. The number in the cells indicates the number of studies. The rank percentile of SUV39H2 in the analyses determines the cell color. Red color denotes for reduction of expression blue color for overexpression. Panels A–C were prepared using COSMIC (http://cancer.sanger.ac.uk/cosmic/), panel D was generated with Oncomine (http://www.oncomine.org/). Similar figures and tables can be easily generated online for each PKMT using the most recent data updates.

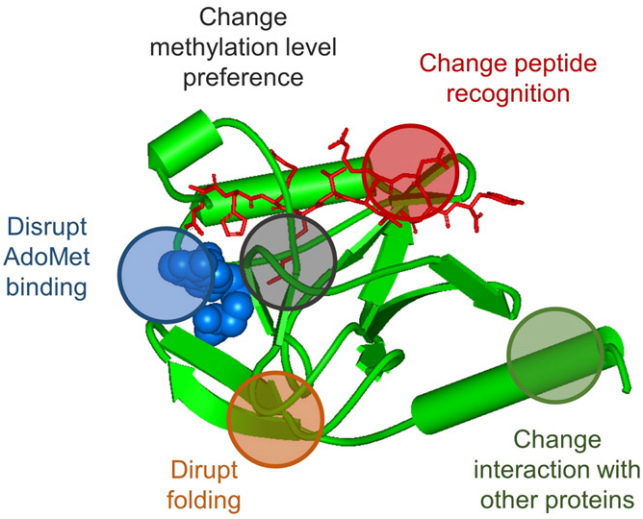

Fig. 3. Potential effects of somatic cancer mutations in PKMTs. The mutation may interfere with AdoMet binding or protein folding, which will lead to the loss of activity. It may alter the methylation level preference of the PKMTs that represents a gain of function effect. In addition, it may change the interaction with other proteins, which may affect the regulation or targeting of the PKMT. Finally, mutations may alter the recognition of the target peptide leading to either loss of the methylation of the original substrate and eventual methylation of novel substrates.

the final methylation state of the substrate lysine in several PKMTs [55]. Using in vitro enzyme assays with reconstituted PRC2 complexes, it was shown that the Y641 mutations result in an increase in trimethylation activity of PRC2 using mono- and dimethylated substrates while the activity towards unmodified peptides was reduced [54,56,57]. The structures of the wild type and mutant SET domains showed that the hydroxylgroup of Y641 (supported by the carbonyl of A677) forms a hydrogen bond to one proton of the lysine ε-aminogroup, which prevents trimethylation (Fig. 5). Therefore, these mutations are examples of mutations changing the methylation level of the product of a PKMT. Hence, EZH2 overexpression or activating mutations likely have a common carcinogenic mechanism based on the hyper-silencing of differentiating genes or tumor suppressor genes.

The dominant mode of action of these hyperactive variants suggests that allele-specific EZH2 inhibitors could be a future therapeutic strategy for tumors containing this mutation. Indeed, a selective inhibitor of EZH2 was developed that reduced H3K27 methylation in various lymphoma cells and initiated apoptotic cell death in heterozygous Tyr641 or Ala677 mutant cells, with minimal effects on the proliferation of wild-type cells [58]. However, EZH2 inactivating mutations were identified as well [59] and overall EZH2 mutations do not show an elevated sense score (Fig. 1B). This finding illustrates that understanding of the mechanism of cancer mutations in PKMTs is an important step for targeted and individualized cancer treatment, for example in the case of EZH2 to decide, whether an EZH2 inhibitor might be a therapeutic option (as in cancers with activating EZH2 mutations) or not (as in cancers with loss-of-function mutations in EZH2).

2.2. The MLL family of PKMTs

The members of the mixed lineage leukemia (MLL) family of PKMTs are the mammalian homologs of the *Drosophila* Trithorax proteins, which specifically methylate lysine 4 of the histone H3 protein and regulate gene transcription during embryogenesis and development [60]. The MLL family consists of several members. In humans, it includes the MLL1-5 proteins and also SET1A and SET1B. A large confusion exists in the nomenclature of MLL proteins, we follow here the nomenclature described by Bögershausen et al. [61]. Like EZH2, MLL proteins function in the context of large protein complexes that in this case include WDR5, ASH2L and RBP5 as core members, which are essential for the complete methyltransferase activity of MLL proteins. The deletion or truncation of different MLL proteins in mice leads to distinguished phenotypes, which indicates that the proteins are not redundant but instead they have specific cellular functions [60]. MLL1 (KMT2A) and MLL4 (KMT2B) are majorly responsible for H3K4me3 at the promoters of genes and regulation of gene expression. In contrast, MLL3 (KMT2C) and MLL2 (KMT2D) majorly introduce a single methyl group

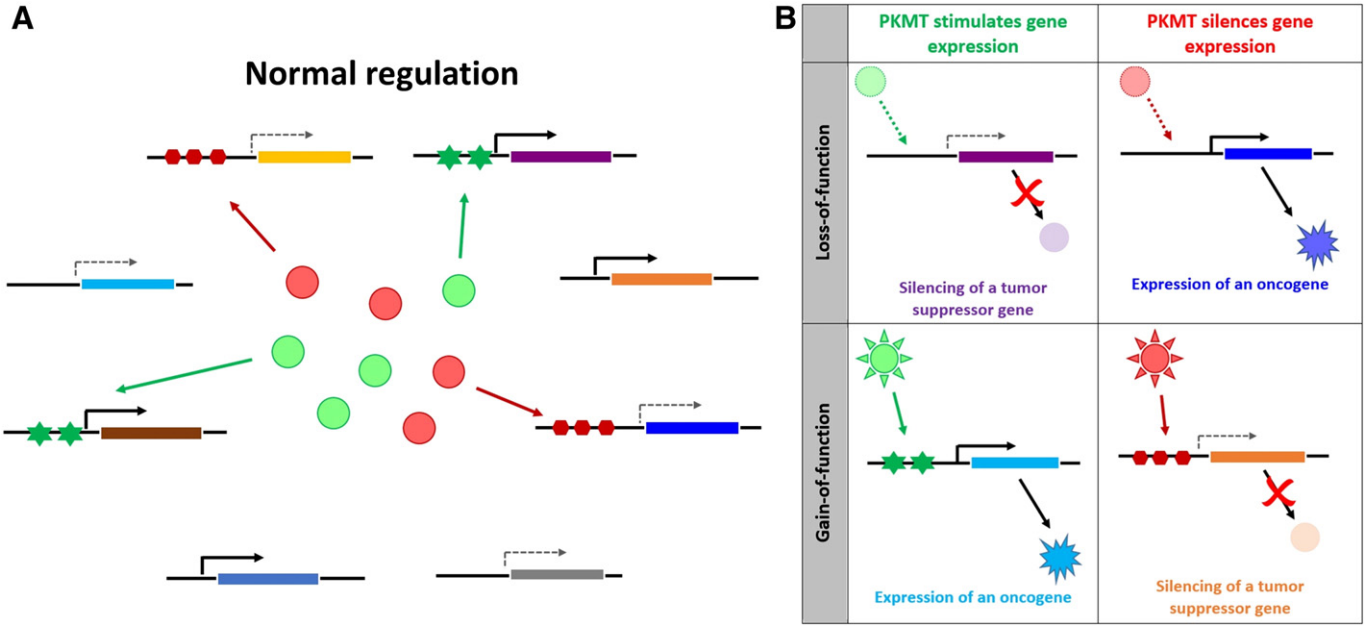

Fig. 4. Schematic drawing of the potential effects of loss-of-function and gain-of-function mutations in PKMTs. A) Schematic picture of "normal" regulation of gene expression, in which some gene promoters carry activating marks (green stars) introduced by corresponding PKMTs (green circles), other genes are marked with repressive modifications (red hexagons) introduced by repressive PKMTs (red circles). These modifications (and other regulatory instances) result in expression of some genes (here: purple, orange, blue and brown), while other genes are repressed (here: yellow, dark blue, gray and light blue). B) Somatic mutations in PKMTs may inactivate them ("loss-of-function") or make them hyperactive or mis-targeted ("gain-of-function"). Depending on the effect of the wild type PKMT on gene expression, this may lead to the overexpression of oncogenes (light and dark blue) or loss of expression of tumor suppressor genes (purple and orange).

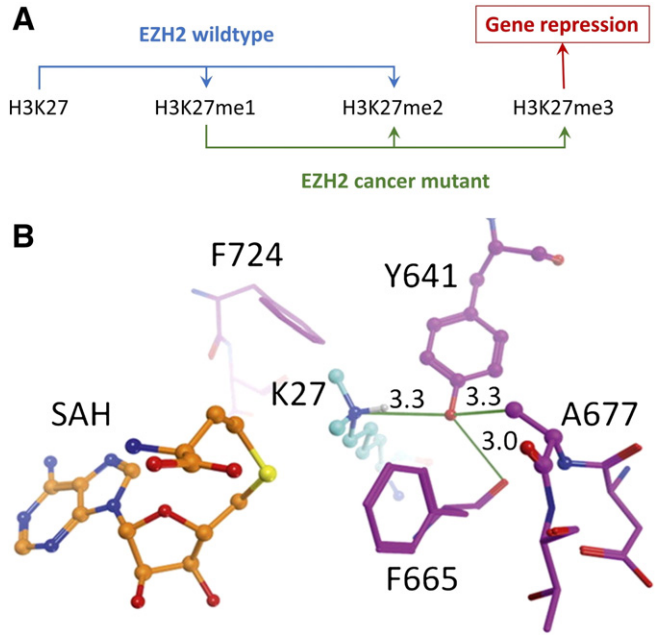

Fig. 5. Mechanism of EZH2 mutations at Y641 and A677. A) The mutations change the substrate preference and final methylation state of the target lysine, leading to the excessive generation of H3K27me3. B) The structure of the SET domain of EZH2 shows that the hydroxyl group of Y641 forms a hydrogen bond to the ε-amino group of K27, which prevents trimethylation. Reproduced from McCabe et al. [57].

on lysine 4 of H3 at the enhancers and promoters of target genes. Recently it has been shown that MLL3 and MLL2 can even repress certain genes [62]. No methyltransferase activity has been reported for MLL5 (KMT2E) so far [63]. The SET1A and SET1B proteins are mammalian orthologs of yeast Set1 and they contribute to the bulk of H3K4 trimethylation in mammals.

2.2.1. The role of MLL PKMTs in cancer

MLL1 (KMT2A) and MLL4 (KMT2B) specifically methylate H3K4, their isolated SET domains monomethylate H3K4 but in complex with WDR5, ASH2L and RBP5 they trimethylate the substrate lysine residue [64]. The MLL1 protein contributes to only about 5% of the global H3K4 trimethylation, nevertheless it is majorly responsible for the regulation of HOX genes [65]. MLL1 undergoes numerous chromosomal translocations with more than 60 different partner genes that are found in several human acute leukemia. AF4, AF9, AF10 and ENL are reported to form fusion partners in more than 75% of MLL associated leukemia, these fusion genes are sufficient to transform normal cells to leukemic cells [66–68] (Fig. 6). In these chromosome translocations, the N terminus of MLL1 is fused in frame with the partner proteins, such that the fusion proteins retain the DNA binding and targeting domains of MLL1, but they lose the C-terminal catalytic SET domain. Aggressive leukemia in both children and adults with MLL1 gene translocations show high expression of HOX genes, which are well-characterized MLL1 target genes [69]. MLL1 knockdown affects cell cycle regulating genes especially cyclin A, cyclin B and p57, which results in a cell cycle arrest in the G2/M phase and apoptosis in cultured cells. However, malignant cells were observed to be more sensitive to MLL1 knockdown than the normal cells, which might be due to the essential requirement of the MLL1 gene for the high proliferation of malignant cells. Consequently, knockdown of MLL1 in a xenograft mouse tumor model was shown to suppress tumor growth [70]. In summary, MLL1 is an oncogenic protein, it is either overexpressed in tumors or it forms fusion partners with other genes and by both mechanisms it enhances the expression of HOX genes.

MLL3 (KMT2C) and MLL2 (KMT2D) share a high degree of similarity in the amino acid sequence and domain structure. They mainly deposit H3K4 monomethylation at enhancers of genes. MLL3 is often deleted in myeloid leukemia [71] and inactivation of MLL3 in mice leads to epithelial tumor formation, which suggests that the methyltransferase activity of MLL3 suppresses tumorigenesis [72]. The MLL3 protein expression was also decreased by at least two fold in almost half of the breast cancer patients when compared to the matched normal tissue, but the expression changes did not correlate with the tumor progression and the pathological effects of changes in the MLL3 expression in breast cancer are still ambiguous [73]. In addition, in colorectal cancer cell lines the MLL3 promoter is hypermethylated, which results in the repression of MLL3 protein expression [74]. Taken together, these findings indicate that MLL3 has a tumor suppressor role in several cancers.

2.2.2. Somatic cancer mutations in MLL PKMTs

MLL3 (KMT2C) and MLL2 (KMT2D) are frequently mutated in cancers (Fig. 1A) and both show a high proportion of loss-of-function mutations (Fig. 1B), suggesting that their inactivation is an important step in carcinogenesis. In early studies, MLL3 somatic mutations were found in glioblastoma [75] and colorectal cancer samples [74,76]. A study using a cohort of breast cancer samples identified somatic frame shift mutations in the MLL3 protein that led to a truncation of the majority of the protein in addition to missense mutations [73]. The known somatic mutations are spread over the entire protein including the PHD fingers and SET domain. Although several mutations were identified in various tumors, their pathological effects are not known. MLL2 is frequently mutated in various cancers as well [56,77]. A study with 89 non-Hodgkin lymphoma (NHL) tumor cell lines, identified 78 somatic mutations distributed across the MLL2 protein, the majority of which were heterozygous. Of the total mutations, 37% were nonsense mutations, 46% were deletions that altered the reading frame, 8% were point mutation at splice sites and 9% were non-synonymous leading to amino acid substitution. Furthermore, somatic MLL2 mutations were identified in 89% of follicular lymphoma patients, 32% of the diffuse large cell B lymphoma (DLBCL) patients and in 59% of DLBCL cell lines. The majority of the MLL2 somatic mutations were inactivating, because they either disrupt the reading frame or are nonsense mutations. This indicates that the MLL2 plays a tumor suppressor role. Somatic inactive mutations of MLL2 were observed on a single copy of a gene suggesting that MLL2 acts as a haploinsufficient tumor suppressor [16]. A study with medulloblastomas also identified mutations in the MLL2 and MLL3 proteins. The majority of these were nonsense mutations resulting in truncated protein products lacking the C terminal SET domain, which again suggest that the tumors might be due to the impaired methyltransferase activity of MLL2 and MLL3 [78].

2.3. The DOT1L PKMT

Dot1 was initially identified in a genetic screen aiming to identify proteins involved in telomeric silencing in yeast [79] and the yeast enzyme [80,81] and its human homolog (called DOT1L) [82] were later demonstrated to methylate K79 of histone H3 which is located in the core structure of histone H3. DOT1L is a unique histone lysine methyltransferase, because unlike other enzymes from this class, DOT1L does not contain a SET domain. Instead its active sites is located in a Rossman-fold domain [83], also found in other class I methyltransferases, like protein arginine and DNA methyltransferases [84], and few other PKMTs [85]. It was shown that yeast DOT1L adds methyl groups to the lysine residue in a nonprocessive manner [86] and it specifically methylates nucleosomes while free histones and peptides are poor substrates. H3K79 methylation is associated with active gene transcription and it is also involved in cell cycle regulation [87]. H3K79me3 and me2 are majorly localized in the coding regions of active genes, whereas H3K79me1 is broadly distributed across the coding region [88]. DOT1L mediated H3K79 methylation is important for embryonic development and a germline knockout of the mouse DOT1L homolog causes embryonic lethality [89]. DOT1L is the sole enzyme that methylates H3K79

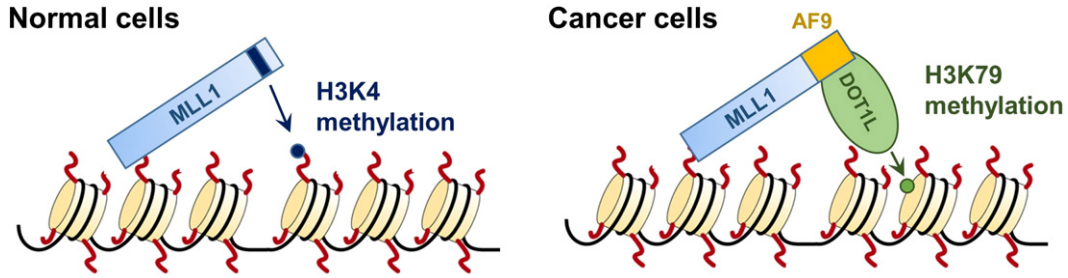

Fig. 6. Schematic picture showing the tumor promoting effect of MLL translocations via recruitment of DOT1L. In normal cells, the MLL1 protein binds to its target genes and introduces H3K4 methylation via its catalytic SET domain (highlighted in dark blue). In the tumor cells MLL1 forms fusions with other protein like AF9, which leads to the loss of the SET domain. The fusion partner interacts with the DOT1L PKMT, which leads to H3K79 methylation and aberrant expression of MLL1 target genes.

indicating that it is the main target protein in diseases related to the aberrant H3K79 methylation [68].

2.3.1. The role of DOT1L in cancer

During the last years several reports have demonstrated that DOT1L is linked to MLL mediated leukemia [68,90]. As described above, 70% of the infant leukemia patients were found to have MLL translocations, in which the N-terminal part of MLL containing its DNA recognition motifs was expressed as a fusion protein with several partner proteins, like AF10, AF9, AF4, or ENL [87,91]. These fusion proteins retain the MLL DNA binding and chromatin targeting domains, which resulted in an altered MLL target gene regulation depending on the fusion partner. Interestingly, it was found that these fusion proteins physically interact with the DOT1L enzyme either directly or indirectly, such that the fusion protein recruits DOT1L to the MLL fusion target loci, which causes aberrant H3K79 trimethylation and can initiate and maintain gene expression (Fig. 6). Thus, DOT1L is an example in which the mistargeting of a PKMT causes methylation at aberrant genomic loci. H3K79 trimethylation plays a crucial role in the regulation of HOX genes such as Hoxa9, Hoxa7 and Meis1, which are overexpressed after aberrant H3K79 methylation in leukemic cells.

By rational drug design and genetic approaches, it was shown that either inhibition of the activity of DOT1L or loss of the DOT1L protein has dramatic effects on the expression of HOX genes. Daigle et al. discovered a competitive inhibitor for AdoMet, the methyl group donor of all known PKMTs, which is specific for DOT1L and selectively inhibits H3K79 methylation [92]. This inhibitor blocked the expression of leukemogenic genes and it selectively killed leukemic cells with MLL translocations with relatively small effect on non-MLL leukemic cells. This result shows that the methyltransferase activity of DOT1L plays a central role in the MLL fusion mediated cellular transformation and maintenance of transformed state and it is a very important drug target with promising initial results [68,90,93,94]. In addition to its pathological function in MLL mediated leukemia, H3K79 hypermethylation was observed in lung cancer cell lines, A549 and NCI-H1299 and lung cancer tissues. Down regulation of DOT1L in lung cancer cell lines blocked the proliferation of cells, which further supports the oncogenic role of DOT1L [95].

2.3.2. Somatic cancer mutations in DOT1L

Several somatic cancer mutations in DOT1L have been identified (Fig. 1), but their mechanism has not yet been described. Based on the known role of DOT1L in cancer, a gain-of-function or hyperactivation effect of the mutations is likely.

2.4. The SETD2 H3K36 PKMT

SETD2 was identified as Huntingtin-interacting protein B (HYPB) [96]. Later, studies have uncovered that it can trimethylate H3K36 via its SET domain, leading to its renaming to SETD2 [97]. The SETD2 protein interacts with the Ser2/Ser5 hyperphosphorylated RNA polymerase 2 during transcriptional elongation via its SRI (Set2 Rpb1 interacting) domain, which explains why H3K36 trimethylation is found in the body of actively transcribed genes [98]. SETD2 is solely responsible for all the H3K36 trimethylation in humans, however, H3K36 mono and dimethylation are also introduced by other methyltransferases like NSD1 [99].

2.4.1. The role of SETD2 in cancer

The SETD2 protein interacts with p53 via its C-terminal SET and WW domains and upregulates p53 activity. Knockdown of SETD2 lowered the expression of p53 target genes such as Puma, Noxa, Huntingtin and p21. In addition, SETD2 also increases the stability of the p53 protein by decreasing the HDMT2 Ring finger-type E3 ubiquitin ligase expression, which can trigger the degradation of p53 by ubiquitination [100]. SETD2 mRNA expression was lost in human breast malignant tissue and the expression levels were negatively correlated with the tumor stage [101]. These data suggest that SETD2 can be used as a pathogenic marker for the breast cancer and SETD2 acts as a tumor suppressor gene [102].

2.4.2. Somatic cancer mutations in SETD2

SETD2 is among the PKMTs with the highest frequency of somatic cancer mutations (Fig. 1A). In agreement with the findings that it acts as tumor suppressor gene, an enrichment of nonsense and frameshift mutations has been observed (Fig. 1B), which is indicative of a loss-of-function mechanism. The SETD2 protein is mutated in high grade gliomas (HGGs): in an analysis of 73 patients with pediatric HGGs, SETD2 mutations were observed in 15% of them and in another cohort of 65 patients with adult HGGs 8% had mutations in the SETD2 protein. However, no mutations were observed in low-grade diffuse gliomas. Gliomas with disruption of SETD2 showed a considerable decrease in H3K36 trimethylation, indicating that the SETD2 mutants negatively affect the activity of the enzyme [103]. SETD2 truncating mutations are majorly found in the C-terminus, which either leads to the loss of the SET or SRI domains; the former leads to loss of PKMT activity and the latter disrupts the interaction with the phosphorylated RNA polymerase II. SETD2 mutations are often observed in leukemia as well, in one study 12% of the acute lymphoblastic leukemia (ALL) patients had mutations in the SETD2 protein, which included loss of function either due to frameshift or nonsense mutations [104]. The SETD2 mutations in ALL patients are not entirely exclusive, they typically have at least one other genetic aberration. Loss of the SETD2 H3K36 methylation activity also contributes to the development of MLL rearranged leukemia, a higher frequency of SETD2 mutations was observed in MLL rearranged leukemia patients (22%) compared to leukemia patients from non-MLL rearrangements (4.6%) [105]. Sequencing data of kidney cancer or clear cell renal cell carcinoma (cRCC) tumor cells identified several missense mutations in the SETD2 protein as well. The SETD2 D1616N mutation that is located in the SET domain of the protein has been identified in the cRCC cell lines which show a considerable loss of H3K36 trimethylation, similarly as other cRCC tumor cell lines containing mutations in the SETD2 protein [106]. All these results document that the loss of SETD2 function plays a pivotal role in the initiation and

progression of cancer, and somatic cancer mutations of SETD2 are examples of loss-of-function.

2.5. The NSD family of PKMTs

The Nuclear Receptor Binding SET Domain Containing Protein 1 (NSD1, KMT3B) is a 2696 amino acids long protein containing a catalytic SET domain, in addition to several PHD domains and two PWWP domains. The SET domain has been initially found to introduce H3K36 mono- and dimethylation in vivo and in vitro [107] and the other domains mediate the interaction with chromatin and other proteins [107–109]. Besides NSD1, the NSD PKMT family consists of two other proteins: NSD2 (WHSC1/MMSET) [110,111] and NSD3 (WHSC1L1) [112]. The NSD PKMTs were reported to methylate multiple lysine residues on histone proteins including H3K4, H3K27, H3K36, H4K20 and H4K44 [113–118] and the p65 non histone protein [36], but the methylation of some of these targets is controversial [118,119]. For NSD1, we recently found that it has its highest activity in methylation of K168 on the H1.5 protein [119]. Defects in the NSD family of proteins are implicated in several diseases. Mutations and deletions in NSD1 lead to the SOTOS syndrome [120] and NSD2 is deleted in the Wolf–Hirschhorn syndrome [121].

2.5.1. The role of NSD PKMTs in cancer

An increasing number of reports document defects in NSD family members in several cancers: NSD1 is associated with acute myeloid leukemia and multiple leukemia, NSD2 protein is linked to prostate cancer and myeloid leukemia and NSD3 is associated with breast cancer and lung cancer [122]. Protein expression studies with 3000 different tumor samples revealed higher NSD2 protein expression when compared to normal tissue. The NSD2 protein expression positively correlated with the progression and advanced tumor stage. In addition, chemotherapy treatment of neuroblastoma cancers decreased the expression levels of NSD2, which suggest NSD2 as a therapeutic target for neuroblastoma [123]. Depletion of NSD2 in a xenograft mouse tumor model, showed a decrease of H3K36me2 and also a decrease of tumor growth [124]. Moreover, NSD proteins form fusion partners via chromosomal translocations in several cancers including NUP98-NSD1 or NUP98-NSD3 generated by the fusion of the nucleoporin gene (NUP98) to either the NSD1 or NSD3 genes, which are found in acute myeloid leukemia. The fusions enhance HOXA5-A10 gene expression via increasing H3K36 dimethylation [125]. Chromosomal t(4; 14) translocations involving NSD2 were observed in multiple myeloma patients. Higher levels of NSD2 protein expression were noticed in 33% of myelomas in a t(4; 14) positive cohort when compared to normal plasma cells [123]. The cells with high levels of NSD2 contained enriched H3K36me2 and lower levels of H3K27 trimethylation and they showed a change in the distribution of H3K36me2, which is enriched in gene bodies in normal cells, but dispersed in the NSD2 overexpressing cells. In summary, the deregulation of NSD proteins plays a causative role in cancers and the histone methyltransferase activity of these proteins is critical for promoting malignant growth (Fig. 7).

2.5.2. Somatic cancer mutations in NSD enzymes

Recent genomic sequence data of cancer cell lines revealed somatic mutations in NSD enzymes (Fig. 1A), which especially in the case of NSD2 suggest a gain-of-function mechanism (Fig. 1B). The heterozygous NSD2 E1099K mutation was recurrently found in several tumors including chronic lymphocytic leukemia (CLL), hypodiploid ALL and lung adenocarcinoma. E1099 resides in the SET domain of NSD2 and it is conserved in all the three NSD proteins, but not in the other histone lysine methyltransferases. The mRNA and protein levels in the NSD2 E1099K mutant cell lines are comparable with the cell lines, but the mutant cell lines exhibit an increase in global H3K36me2 levels and concomitant decrease of H3K27me3 levels and changes in the distribution of H3K36 methylation (Fig. 7). In agreement with this, the NSD2-E1099K mutant showed an increased activity for in vitro methylation of recombinant nucleosomes as compared to NSD2 wild type. However, different from the EZH2 mutations described above, the E1099K mutation enhances the methyltransferase activity of NSD2 without altering the product specificity. In addition, ALL tumors also harbor D1125N mutations in NSD2, this variant is also associated with higher H3K36me2 methyltransferase activity [124]. The effect of these activating mutations is comparable to the chromatin alterations observed in the NSD2 overexpressing t(4; 14) positive cells. Hence, the somatic cancer mutations in NSD2 are examples of a gain-of-function similarly as the overexpression of NSD2. These observations indicate that NSD2 has a vital role as oncoprotein [126].

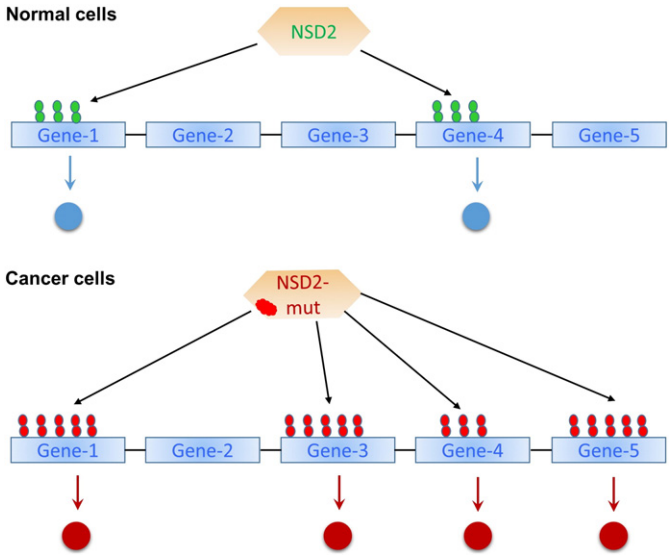

Fig. 7. Schematic picture showing the tumor promoting effect of NSD2 overexpression or activating NSD2 cancer mutations. In normal cells, NSD2 protein introduces H3K36 methylation at its target genes. In tumor cells with increased NSD2 activity, hypermethylation of H3K36 at non-target genes occurs, which can lead to the expression of oncogenes.

2.6. The SMYD family of PKMTs

The SMYD PKMT protein family consists of five members named SMYD1–5. SMYD proteins are not well characterized and are grouped based on their similar domain architecture. The proteins contain a split SET domain which carries an inserted MYND (Myeloid, Nervy and DEAF-1) domain. The MYND domain is responsible for protein–protein interactions and the split SET domain contains the important catalytic elements as in other SET domain enzymes [127]. Of the five SMYD proteins, SMYD2 and SMYD3 are best characterized. SMYD1 [128] and SMYD3 [129] were identified as H3K4me3 methyltransferases, while SMYD2 has been reported to monomethylate several lysine residues on histone and non-histone proteins. It was initially shown to methylate H3K36 [130] but later it was reported that the interaction with HSP90a changes its specificity towards H3K4 [131]. Additional studies showed that Smyd2 also methylates K266 of estrogen receptor alpha [132], K370 of p53 [133] and K810 and K860 of the retinoblastoma (RB) protein [134,135].

2.6.1. The role of SMYD PKMTs in cancer

Methylation of non-histone targets by SMYD2 has clear connections to cancer. The methylation of p53 at K370 by SMYD2 impairs the tumor suppressor activity of p53, such that overexpression of SMYD2 has a tumor promoting effect. Similarly, monomethylation of the Rb protein at K810 enhances Ser807 and 811 phosphorylation of Rb and leads to its dissociation from E2F, which enhances its transcriptional activity and promotes cell cycle progression [135]. SMYD2 is significantly overexpressed in several cancers. For example, the SMYD2 mRNA levels

were reported to be almost 8 fold increased in leukemia cases, when compared with normal bone marrow controls and patients with high SMYD2 expression showed lower survival rate [136]. Similarly, SMYD2 protein over expression was also identified in esophageal squamous cell carcinoma (ESCC). Knockdown of SMYD2 in ESCC cell lines with overexpression of SMYD2 led to the suppression of proliferation due to a G_0–G_1 arrest, suggesting that SMYD2 inhibitors might be good cancer drugs in these cases. In addition, overexpression of SMYD2 both at the mRNA and protein level was reported in the bladder cancer samples [135]. In summary overexpression of SMYD2 is frequently observed in several cancer cell lines and it is correlated with lower survival rate indicating that SMYD2 acts as an oncoprotein [137].

Alterations in the expression levels of other SMYD proteins were also recurrently observed in cancers. Silent or very weak SMYD4 gene expression has been reported in breast cancer cells. This was correlated to higher expression of the platelet derived growth factor receptor α (Pdgfr-α). In several mammalian cancers, Pdgfr-α is an oncogene, which is involved in the proliferation and survival of variety of tumors. Re-expressing SMYD4 in breast cancer cell lines repressed the Pdgfr-α gene, suggesting that SMYD4 acts as potential tumor suppressor at least in this cellular model system [138]. SMYD3 was initially reported as an H3K4me3 methyltransferase [129] and later to trimethylate H4K20 [139]. However, Kruger and colleagues showed that SMYD3 methylates H4 protein at K5 instead of K20 [140]. Therefore, the methylation site(s) of SMYD3 are still ambiguous. SMYD3 is an oncogenic protein: elevated levels of SMYD3 protein expression were observed in colorectal carcinoma (CRC), hepatocellular carcinoma (HRC) and breast cancer cells, whereas weak or no expression was observed in non-cancerous cells of other tissues. In agreement with this, suppression of SMYD3 significantly reduced the growth of CRC and HRC cells [129]. In breast cancer cell lines, a truncated SMYD3 protein lacking the first 34 amino acids was observed as well, which was shown to have a higher activity than the full length SMYD3 [141]. Moreover, it has been shown that SMYD3 up-regulates the expression of the oncogene matrix metalloproteinase (MMP-9), which is involved in the tumor progression and metastasis by stimulating cell migration, tumor invasion and angiogenesis. Suppression of SMYD3 reduces the MMP9 gene expression supporting its oncogenic property [142]. Recently, it has been shown that SMYD3 methylation of additional non-histone substrates is a key event in Ras-driven carcinomas. SMYD3 methylates the MAP3K2 kinase at K260, which increases MAP kinase signaling and leads to the formation of Ras driven carcinomas. In addition, MAP3K2-K260 methylation inhibits binding of PPP2 phosphatase complex, which is a cellular phosphatase and a key negative regulator of MAP kinase signaling pathway [143]. Altogether these data suggest that the SMYD proteins play vital roles in regulating the expression of oncogenes in several cancers with SMYD4 acting as tumor suppressor while SMYD3 and SMYD2 function as oncogenic factors.

2.6.2. Somatic cancer mutations in SMYD PKMTs

Recent genomic sequencing in cancer patients revealed several somatic mutations in SMYD proteins [144–146] with SMYD1 showing the highest proportion of mutations (Fig. 1A). The somatic mutations in SMYD PKMTs include missense and nonsense mutations as well as insertions and deletions. Interestingly, the mutational patterns are indicative of a gain-of-function for SMYD1 and in particular SMYD4, which is not in agreement with the finding that it acts as tumor suppressor gene in breast cancer cells as described above, suggesting that the role of SMYD4 is complex. Since SMYD proteins methylate several histone and non-histone substrates that may play roles in gene expression regulation and tumor formation, it is important to understand the pathogenic mechanism of these somatic missense mutations in tumor cells. Hyperactive somatic mutations could have the same effect as the overexpression of SMYD2 or SMYD3 in cancer cells and they may, for example, aberrantly increase the lysine methylation of tumor suppressor p53 and decrease its tumor suppressor function. Additionally, it will be interesting to see whether SMYD protein mutations may change the product specificity of the PKMT. Given the wide spectrum of non-histone proteins methylated by members of the SYMD family of PKMTs it is also likely that some of the somatic cancer mutations will alter the substrate spectrum of the enzymes (Fig. 8), although such an effect still awaits experimental validation.

3. Conclusion

Proteomic and genomic approaches have allowed addressing several mechanisms of carcinogenesis origin, but cancer is continuously confronting us with the new challenges. It has been known that the cancer arises as a result of gene mutations and clonal proliferation of cells containing growth promoting alterations [18]. The specific mutations identified in defined cancer subtypes have important diagnostic significance. For instance, Janus kinase 2 mutation (JAK V2617F) is present in many myoproliferative neoplasma and polycythemia vera and now this mutation is used as diagnostic marker for this condition [22]. In addition, epigenetic alterations were recognized to play important roles in tumor formation [19]. The recent discovery and characterization of somatic tumor mutations in epigenetic enzymes connect these two processes, because in these cases the genetic changes drive the appearance of epigenetic alterations [147]. One large class of epigenetic enzymes is PKMTs and the investigation of the role of somatic mutations in these enzymes in carcinogenesis is an emerging approach to the mechanistic understanding of tumor formation and progression.

While the mechanism of loss-of-function mutations can be deduced from the function of the corresponding gene product, this is not necessarily true for gain-of-function mutations, which in the case of PKMTs might have diverse pathogenic effects: they might enhance the enzymatic activity of the PKMT, increase the stability of the protein or alter its cellular targeting. Somatic mutations in PKMTs might also change their association with interacting partners, or recruit the PKMT to specific genetic loci resulting in the altered expression of particular oncogenes or tumor suppressor genes. The higher activity of a PKMT might lead to abnormal methylation of histones at particular genes, which either enhance or inhibit the transcription of oncogenes or tumor suppressor genes. Mutations in the SET domain of PKMTs might change their peptide sequence specificity leading to the methylation of novel targets or they may change the product specificity leading to a change in the final methylation state of the target lysine residues. In most cases, the mechanistic consequences of somatic mutations in PKMTs

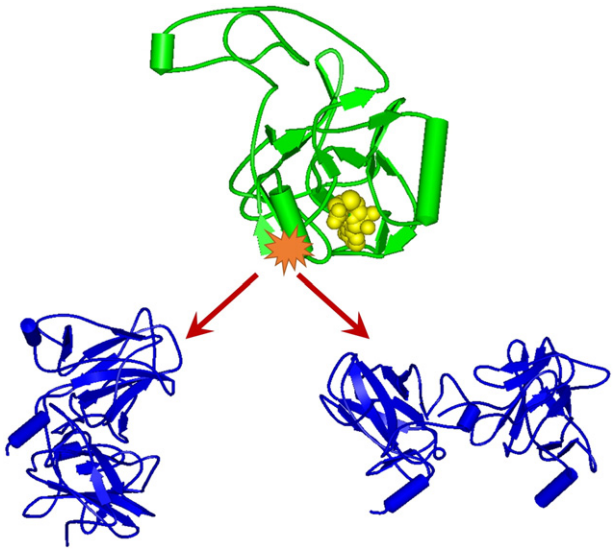

Fig. 8. Schematic picture showing the hypothetical methylation of abnormal target proteins (blue) by a PKMT (green) caused by a somatic cancer mutation (indicated by an orange star), which alters the substrate specificity or general activity of the PKMT.

are not yet known and careful biochemical studies will be needed for their elucidation. The study of the pathomechanism of somatic cancer mutations in PKMTs holds great promises for several reasons:

1) It will be important in the future to investigate the effects of somatic mutations in order to develop individualized cancer treatments. For example, inhibitors developed against specific PKMTs might assist treatment of cancers with hyperactive PKMT mutants, but these inhibitors cannot be used to treat the cancers caused by the loss of PKMTs activity. This makes the investigation of the pathomechanism of somatic cancer mutations very relevant for the development of novel more causative and individualized tumor therapies.
2) The investigation of somatic cancer mutations is also imperative for the general understanding of the carcinogenic process. Cancers are regularly caused by changes in the activity of critical proteins, like PKMTs as illustrated in this review. This might be due to the overexpression or loss of expression of a specific enzyme or a particular post-translational modifications (PTM), which render the enzyme either hyperactive or inactive. However, these effects are often transient and the expression level or PTM pattern of a PKMT found in tumor tissues or even in tumor cell lines might not always reflect the situation in the critical phase of cellular transformation. The effects of somatic tumor mutations of PKMTs often resemble the effects of overexpression, loss of expression or misregulation of the corresponding enzymes but, different from expressional changes and post-translational modifications, the somatic cancer mutations are stable, which means that they can be studied in tumor samples and cell lines much more easily. Moreover, the effects of somatic mutations can be examined with in vitro experiments, which is much more difficult with changes of expression levels or modification states. Therefore, studying and understanding of the mechanism of somatic cancer mutations will help to unravel the role of the corresponding PKMT even in cases not involving mutations.
3) The screening for somatic cancer mutations also holds great promises in clinical practice, since the detection of mutations occurs at DNA level, which can be done from small samples of cancer tissue. In contrast the detection of expression changes or aberrant PTMs needs RNA and proteomic methods, which are much more demanding with respect to sample size and sample treatment. Furthermore, simple patient blood samples can be used as normal controls in mutational screenings, while the acquisition of matching "normal" tissue for comparison is sometimes very difficult in expression studies and proteomics work.

In summary, we argue here that the investigation of the pathomechanism of somatic cancer mutations is an important and urgent challenge in biomedical research. For this, relevant assay systems need to be established to investigate the properties of PKMTs and their cancer mutants in vitro and in cellular settings. These studies will also pave the way towards the generation of novel and more potent and specific PKMT inhibitors and guide their later clinical use.

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