



# SETting the Stage for Cancer Development: SETD2 and the Consequences of Lost Methylation

Catherine C. Fahey<sup>1</sup> and Ian J. Davis<sup>1,2</sup>

<sup>1</sup>Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7295

<sup>2</sup>Departments of Genetics and Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7295

Correspondence: [ian\\_davis@med.unc.edu](mailto:ian_davis@med.unc.edu)

The H3 lysine 36 histone methyltransferase *SETD2* is mutated across a range of human cancers. Although other enzymes can mediate mono- and dimethylation, *SETD2* is the exclusive trimethylase. *SETD2* associates with the phosphorylated carboxy-terminal domain of RNA polymerase and modifies histones at actively transcribed genes. The functions associated with *SETD2* are mediated through multiple effector proteins that bind trimethylated H3K36. These effectors directly mediate multiple chromatin-regulated processes, including RNA splicing, DNA damage repair, and DNA methylation. Although alterations in each of these processes have been associated with *SETD2* loss, the relative role of each in the development of cancer is not fully understood. Critical vulnerabilities resulting from *SETD2* loss may offer a strategy for potential therapeutics.

Large-scale sequencing efforts of human cancers have identified recurrent mutations and deletions in a variety of chromatin regulatory proteins. *SETD2*, a methyltransferase that trimethylates histone H3 at lysine 36 (H3K36me<sub>3</sub>), has been found to be both mutated and deleted in select range of cancers suggesting a role in tumor suppression. In this review, we will discuss the range of *SETD2* mutations and how disruption of this gene could foster the development of cancer.

## ENZYMATIC FUNCTION AND STRUCTURE OF SETD2

*SETD2* (also known as HYPB or KMT3a) is the sole methyltransferase that mediates trimethyl-

ation of histone H3.1 and H3 variants (Fig. 1) (Edmunds et al. 2008). Although *SETD2* shows biochemical evidence of mono- and dimethylation activity, in cells it seems to exclusively mediate trimethylation because *SETD2* silencing results in a near complete loss of H3K36 trimethylation without decreasing mono- or dimethylation levels (Edmunds et al. 2008; Yuan et al. 2009). In higher eukaryotes, other enzymes, including NSD1, WHSC1 (NSD2), and SETMAR, are able to mono- and dimethylate H3K36 (reviewed in Wagner and Carpenter 2012). In contrast, the homolog of *SETD2* in *Saccharomyces cerevisiae*, *Set2*, exclusively mediates all H3K36 methylation states (Strahl et al. 2002). Although extensive genetic experimentation with yeast *Set2* has informed our under-

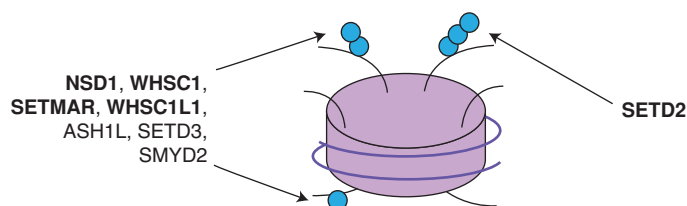
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**Figure 1.** H3K36 methyltransferases. Methyltransferases shown to mono-, di-, and trimethylate H3K36. Those shown in bold have been shown in cell-based assays and/or in vivo. (Figure adapted from data in Wagner and Carpenter 2012.)

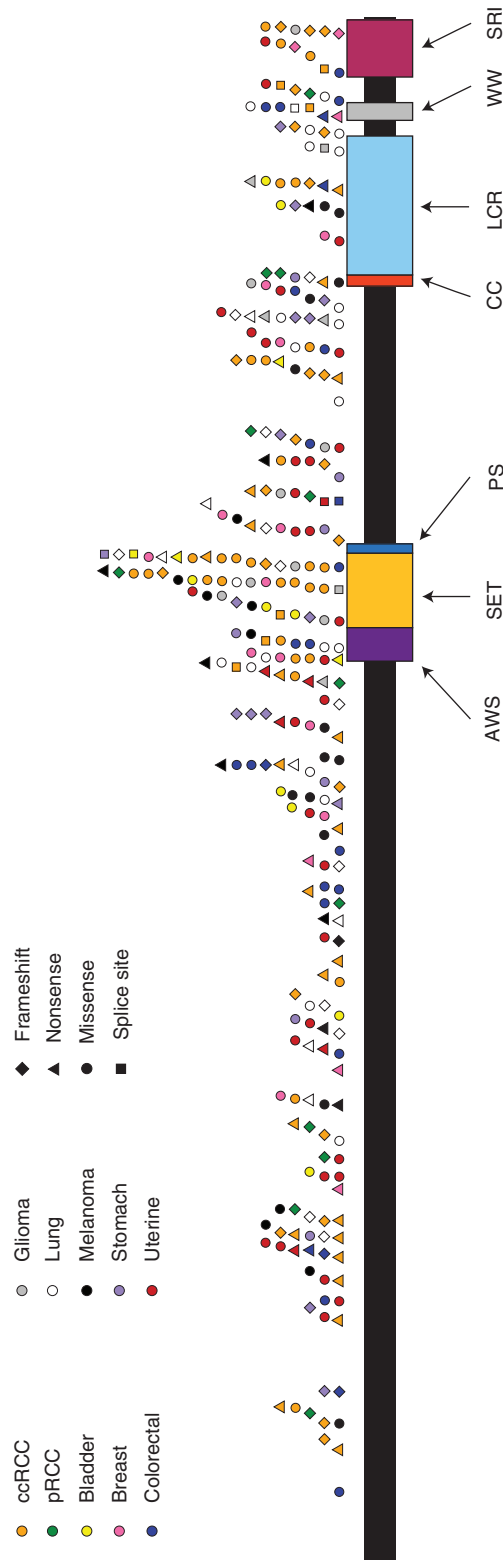
standing of the biochemical properties of critical SETD2 domains as well as the possible roles, the redundancy of mono- and dimethylation activities in humans offers an important caveat when extrapolating results from *S. cerevisiae*.

The functions of SETD2 have been attributed to several domains in the protein (Fig. 2). These domains share sequence homology as well as functional similarity with those in yeast Set2. The methyltransferase activity is mediated by the conserved SET domain (Strahl et al. 2002; Li et al. 2005). SETD2 contains two known protein-binding domains: SRI (Set2 Rpd1 Interacting) and WW. The SRI domain mediates association with the hyperphosphorylated carboxy-terminal domain (CTD) of RNA polymerase II (RNAPII) (Li et al. 2005; Sun et al. 2005; Xiao et al. 2003). In yeast, this interaction is required for H3K36 activity, as deletion of the RNAPII CTD decreases H3K36 methylation levels (Li et al. 2003; Xiao et al. 2003). The WW domain, which precedes the SRI domain, may mediate intramolecular interaction (Gao et al. 2014). Based on the property of WW domain interaction with phosphorylated proteins, this domain could also mediate other protein interactions (Lu et al. 1999). About half of SETD2 consists of a large amino terminal domain that is not shared by yeast Set2 and is of unknown function.

### SETD2 MUTATION IN CANCER

Hundreds of distinct SETD2 mutations have been identified across a wide range of human tumors, including epithelial, central nervous system (CNS), and hematopoietic (Fig. 2; Table 1) (Gao et al. 2013; Cerami et al. 2012). SETD2

mutation was first described in clear cell renal cell carcinoma (ccRCC). In a cohort of 407 ccRCC tumors, truncating mutations were observed in 12 samples (Dalglish et al. 2010). SETD2 mutation was also found in ccRCC cell lines (Duns et al. 2012). In The Cancer Genome Atlas (TCGA) study of ccRCC, SETD2 was the third-most-commonly mutated gene with a prevalence of 15.6%. SETD2 is located on chromosome 3p, which shows near universal loss of heterozygosity in ccRCC (Zbar et al. 1987). Chromosome 3p is also the location of the well-known tumor suppressor von Hippel–Lindau (VHL). VHL expression is lost in most cases of sporadic ccRCC, and germline mutation is associated with a high penetrance of ccRCC (Stolle et al. 1998). Mutations of SETD2 affect the remaining allele, and frequently have a significant impact on gene function. Most mutations tend to be inactivating frameshift or nonsense mutations, although missense mutations in critical domains have been detected (Gerlinger et al. 2012; The Cancer Genome Atlas 2013a; Gossage et al. 2014; Simon et al. 2014). In a study of 128 sporadic ccRCC tumors that specifically examined genes known to be mutated in ccRCC, SETD2 was mutated in ~16% (Gossage et al. 2014). Five frameshift, 10 nonsense, and two splice site mutations were observed. Of three nonsynonymous missense mutations, one altered the SET domain (Gossage et al. 2014). Studies of intratumoral genetic heterogeneity also support a key role for SETD2 loss. Sequencing multiple sites in a single kidney tumor together with metastatic sites identified multiple distinct SETD2 mutations each likely to disrupt function. This convergent tumor evolution suggests that SETD2 mutation



**Figure 2.** Schematic representation of SETD2 and cancer-associated mutations. SETD2 domains: AWS (associated with SET), SET (Su(var)3-9, enhancer-of-zeste, trithorax), PS (post-SET), CC (coiled coil), LCR (low charge region), WW, SRI (Set2 Rpb1 Interacting). Mutation lists were obtained from CBioPortal on February 5, 2016, and separated into cancer types. Duplicates were removed. Mutations are plotted by color (cancer type) and shape (mutation type). ccRCC, Clear cell renal cell carcinoma; pRCC, papillary renal cell carcinoma.

**Table 1.** Cancers associated with SETD2 mutation

Cancer type	Mutation % (total samples)	References
Clear cell renal cell carcinoma	15.6% (418 samples)	The Cancer Genome Atlas 2013a
High-grade glioma	15% (543 samples)	Fontebasso et al. 2013
Uterine carcinosarcoma	13.6% (22 samples)	Jones et al. 2014
Uterine corpus endometrioid carcinoma	9% (240 samples)	The Cancer Genome Atlas et al. 2013
Acute lymphocytic leukemia	12% (125 samples) 10% (94 samples)	Zhang et al. 2012; Mar et al. 2014
Bladder urothelial carcinoma	10.2% (107 samples) 6% (50 samples)	The Cancer Genome Atlas 2014b; Van Allen et al. 2014
Desmoplastic melanoma	10% (20 samples)	Berger et al. 2012
Melanoma	8% (25 samples)	Shain et al. 2015
Cutaneous melanoma	5.5% (91 samples)	Berger et al. 2012; Hodis et al. 2012
Lung adenocarcinoma	9% (230 samples) 5.5% (182 samples)	Imielinski et al. 2012; The Cancer Genome Atlas 2014c
Colorectal adenocarcinoma	8.3% (72 samples) 6.1% (212 samples)	The Cancer Genome Atlas 2012; Seshagiri et al. 2012
Pancreatic adenocarcinoma	8.3% (109 samples)	Witkiewicz et al. 2015
Stomach adenocarcinoma	7% (287 samples)	The Cancer Genome Atlas 2014a
Papillary renal cell carcinoma	7.6% (157 samples)	The Cancer Genome Atlas et al. 2016
Cutaneous squamous cell carcinoma	6.9% (29 samples)	Li et al. 2015

Cancers are selected for which the mutation rate in CBioPortal (Cerami et al. 2012; Gao et al. 2013) exceeded 5% and a publication was available. Indicated mutation rate reflects published results. Additional cancers discussed in the text are also included.

is a critical event for a subset of ccRCCs (Gerlinger et al. 2012). Suggestive of a link with aggressive disease, a lower level of H3K36 trimethylation was observed in tumors in metastases compared with primary tumors (Ho et al. 2015).

*SETD2* mutations have also been identified in multiple other cancers. High-severity *SETD2* mutation was observed in 15%–28% of pediatric and 8% of adult high-grade gliomas (HGGs) (Fontebasso et al. 2013). In contrast, *SETD2* mutations were not identified in low-grade gliomas. In addition, all tumors with *SETD2* mutation were located in the cerebral hemispheres (Fontebasso et al. 2013). However, mutation of *SETD2* was detected in a single diffuse intrinsic pontine glioma, although it co-occurred with an H3.1 K27M mutation, a common feature of these tumors (Wu et al. 2014). Intriguingly, high-grade hemispheric gliomas of children

and young adults are also commonly associated with mutation of histone H3.3 (*H3F3A*) at glycine 34 (Schwartzentruber et al. 2012; Wu et al. 2012). These mutations were nonoverlapping with *SETD2* and were associated with reduced H3K36 methylation (Schwartzentruber et al. 2012; Sturm et al. 2012; Fontebasso et al. 2013). Gliomas also commonly harbor IDH1 mutations, which result in the generation of the 2-hydroxyglutarate (2-HG) oncometabolite (Dang et al. 2009). These mutations are not mutually exclusive with *SETD2* mutation. Although 2-HG inhibits histone demethylases, including those that can act on H3K36, it is not clear whether IDH1 mutation directly affects H3K36me3 (Chowdhury et al. 2011; Xu et al. 2011; Lu et al. 2012; Fontebasso et al. 2013). Overall, these findings indicate that dysregulation of H3K36me3 is a common event in glioma. The finding linking H3.3 mutation with

reduced H3K36 methylation will be discussed in more detail below.

*SETD2* mutations have also been identified in acute leukemias. In a study of early T-cell precursor acute lymphoblastic leukemia (ETP-ALL), approximately 10% showed deletion or high-severity mutation of *SETD2* (Zhang et al. 2012). In a separate study, *SETD2* mutation was detected in approximately 6% of ALL and AML samples (Zhu et al. 2014). Mutation of *SETD2* was more common in both acute lymphoid and myeloid leukemias with mixed lineage leukemia (MLL)-rearrangement compared to acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) with an intact MLL gene. Further supporting *SETD2* loss as a critical event in leukemia development, *SETD2* mutations were commonly nonsense or frameshift, and approximately a quarter of samples carried biallelic *SETD2* mutations (Zhu et al. 2014). A comparison of matched primary and relapsed ALL samples suggested that mutations in epigenetic regulators as a class were more common at relapse, and this included mutations in *SETD2* (Mar et al. 2014). In this study, *SETD2* showed a mutation rate of 5% in a pilot cohort and 12% in a larger validation set. The validation set contained a higher fraction of MLL-rearranged samples possibly explaining the discrepancy in mutation frequency. Suggesting that the importance of *SETD2* mutation is greater in acute leukemias in children, the study of AML by TCGA identified only a single *SETD2* mutation among 191 adult samples (The Cancer Genome Atlas 2013b). The link between *SETD2* loss and MLL rearrangement is provocative since, like *SETD2*, MLL fusion proteins can associate with components of the transcriptional complex, and the combined alterations in these proteins may lead to transcriptional dysregulation (Milne et al. 2010; Yokoyama et al. 2010).

*SETD2* mutations have also been observed at a low frequency in a range of other tumors. 6% of melanoma and chronic lymphocytic leukemia showed *SETD2* alteration (Berger et al. 2012; Lee et al. 2015; Parker et al. 2016). *SETD2* alterations were observed in high-risk, but not low-risk, gastrointestinal stromal tumors (Huang et al. 2015). *SETD2* mutation has also

been described in phyllodes tumors of the breast, but not in breast fibroadenoma (Tan et al. 2015; Liu et al. 2016). Among other genitourinary tumors, *SETD2* mutation is found in 10% of bladder tumors and the papillary subtype of renal cell carcinoma (The Cancer Genome Atlas 2014b; The Cancer Genome Atlas et al. 2016). Although many of these mutations are monoallelic and consequently predicted to lead to haploinsufficiency, mutations are not the exclusive mechanism for modulating *SETD2* activity.

Decreased H3K36me3 has also been observed in the context of nonmutant *SETD2* in ccRCC (Simon et al. 2014). miR-106b-5p, a micro RNA known to regulate *SETD2*, was elevated in a cohort of 40 ccRCC tumor samples; levels of this miRNA inversely correlated with *SETD2* mRNA and protein levels (Xiang et al. 2015). *SETD2* mRNA levels were also decreased in a subset of patients with AML and lymphoma (Zhu et al. 2014).

In addition to alterations in *SETD2*, H3K36me3 can be lost by mutation of the methyl acceptor site in histones or by mutations in neighboring amino acids. Virtually all chondroblastomas harbor a lysine 36 to methionine variant in histone H3.3 (H3.3K36M) (Behjati et al. 2013). Expression of the H3.3K36M mutant in cells led to depletion of all H3K36 trimethylation (Lewis et al. 2013). H3.3K36M binds and directly inhibits the activity of *SETD2* and the dimethylation activity of MMSET (Fang et al. 2016). Mutations in H3.3 at the neighboring G34 residue have been described in almost all giant-cell bone tumors (Behjati et al. 2013) and, as previously mentioned, in high-grade gliomas (Schwartzentruber et al. 2012; Wu et al. 2014). Overall, the common finding of loss or inhibition of *SETD2* across a wide range of cancers suggests the importance of disrupted H3K36 methylation in cancer development.

## TRANSCRIPTION AND RNA SPLICING

Chromatin influences many cellular processes including transcription, replication and DNA damage repair. Many studies have linked *SETD2*



and transcription. In yeast, Set2 and H3K36 trimethylation are associated with gene bodies, and H3K36me3 levels correlate both with the level of transcription and the position in the gene (Krogan et al. 2003; Bannister et al. 2005). H3K36me3 signals are enriched at exons, although the higher levels of nucleosome occupancy at exons may explain this difference (Schwartz et al. 2009). Treatment with a transcription inhibitor causes a decrease in H3K36me3 levels (de Almeida et al. 2011), suggesting that active transcription is necessary for H3K36me3 placement. These data are consistent with the model that Set2 and SETD2 are targeted to elongating RNAPII.

Our understanding of the role of SETD2 in transcription is largely based on studies of Set2 in yeast. In yeast, Set2 partially functions to prevent cryptic initiation, aberrant transcription from internal sites. H3K36 methylation recruits the Rpd3C(S) complex, which includes a histone deacetylase (Carrozza et al. 2005; Keogh et al. 2005), leading to the deacetylation of histones in gene bodies. H3K36 methylation also suppresses of the interaction between histone H3 and the histone chaperone Asf1 (Venkatesh et al. 2012). Preventing the incorporation of new acetylated histones maintains a hypoacetylated state, thereby stabilizing nucleosomes that decreases the chance of a segment of gene being aberrantly recognized by the transcriptional initiation complex as a promoter (Carrozza et al. 2005; Li et al. 2007; Lickwar et al. 2009).

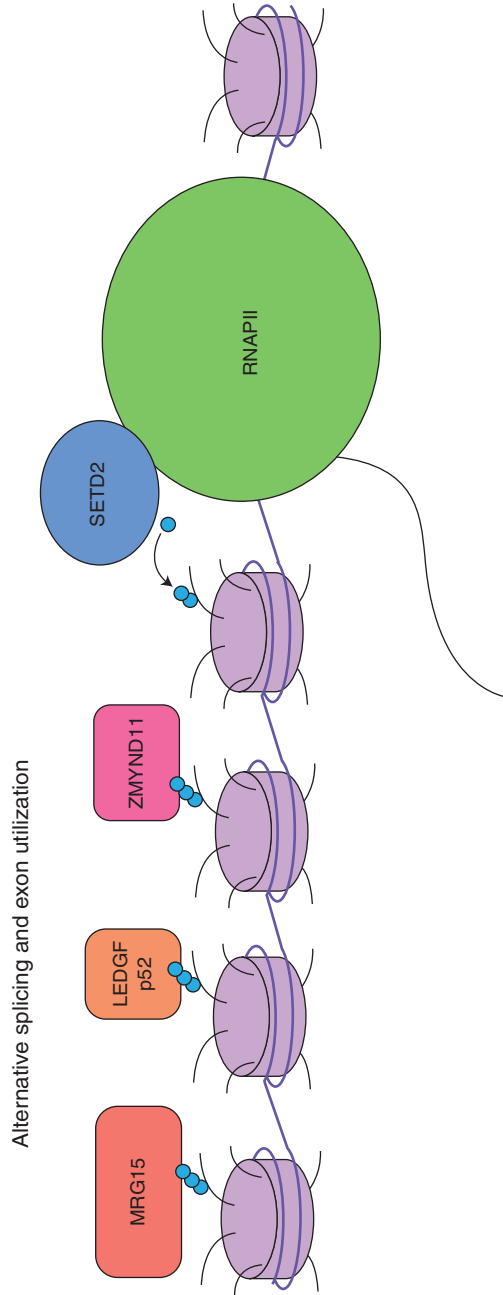
The role of SETD2 in transcription in higher eukaryotes is more complicated. In addition to the separation of methylation activities across multiple enzymes, genes in higher eukaryotes contain introns and are regulated by alternative splicing, and DNA itself can be methylated. H3K36 methylation levels differ based on exon utilization (Kolasinska-Zwierz et al. 2009), with alternatively spliced exons having lower levels of H3K36me3 than those that are constitutively included. Altering SETD2 levels influenced the inclusion of exons in genes known to be alternatively spliced (Luco et al. 2010). Deletion of the splice acceptor site in the  $\beta$ -globin intron leads to shifts in H3K36me3 signal (Kim et al. 2011), and intronless genes have lower levels of

H3K36 trimethylation (de Almeida et al. 2011). Chemical or RNAi-mediated inhibition of splicing decreased H3K36me3 levels. Together these data suggest a close relationship between trimethylation of H3K36 and RNA splicing. Perhaps reflecting aberrant transcription or RNA processing, silencing SETD2 results in mRNA accumulation in the nucleus (Yoh et al. 2008).

Several studies have explored the impact of SETD2 loss on transcription in kidney cancer. Examining primary ccRCC, H3K36me3-deficient tumors show alterations in splicing and evidence of intron retention (Simon et al. 2014). This association was also detected in transcriptomic ccRCC data from TCGA. Similarly, differential splicing and altered exon utilization was observed in *SETD2* knockout cells (Ho et al. 2015). However, no difference in exon usage or intron retention was observed in other studies in which SETD2 was silenced using RNAi (Kanu et al. 2015). SETD2 has also been associated with aberrant transcriptional termination (Grosso et al. 2015). In the absence of appropriate termination, RNAPII complexes can read through into neighboring genes yielding chimeric RNAs.

Several proteins that bind H3K36 methylation offer a link between SETD2 and RNA splicing (Fig. 3). LEDGF (PSIP1) exists as two isoforms, p52 and p75, based on the inclusion of six additional 3' exons (Singh et al. 2000). Both forms contain a PWWP domain, which interacts with di- and trimethylated H3K36. The short form, LEDGF/p52, interacts with proteins involved in alternative splicing (Pradeepa et al. 2012). ZMYND11, also a PWWP domain-containing protein, binds H3.3K36 and associates with regulators of RNA splicing (Guo et al. 2014). MRG15 is a chromodomain-containing protein that binds di- and trimethylated H3K36 (Zhang et al. 2006) and recruits polypyrimidine tract-binding protein (PTB) to alternatively spliced exons (Luco et al. 2010). PTB then binds to silencing elements causing repression of specific exons.

In embryonic stem cells, MRG15 also recruits the lysine demethylase KDM5B to H3K36me3 marked chromatin (Xie et al. 2011). Silencing KDM5B resulted in recruit-



**Figure 3.** Transcription and RNA processing. SETD2 associates with RNAPII to posttranslationally modify nucleosomes. H3K36me3 is directly bound by readers, which mediate RNA processing through downstream effectors.

ment of unphosphorylated RNAPII to intragenic regions marked by H3K4me3, potential sites of cryptic initiation. Knockdown of KDM5B increased levels of unspliced transcripts, possibly reflecting aberrant transcription. Downregulation of SETD2 was also found to be associated with increased RNA abundance at noninitiating exons, potentially indicating transcriptional initiation from these sites (Carvalho et al. 2013). Taken together, the extent to which cryptic initiation in yeast is a function of dimethylation (rather than trimethylation) or that RNA alteration in higher eukaryotic cells results from aberrant splicing (rather than cryptic initiation) are unknown.

Overall, SETD2-mediated histone modification and its interaction with specific binding partners offers an explanation for the variation in transcription and aberrant RNA detected after SETD2 loss. Although intron retention has been shown to be a mechanism of tumor-suppressor inactivation (Jung et al. 2015), how SETD2 loss facilitates tumor development remains unknown.

### CHROMATIN STRUCTURE

Several studies have shown that H3K36me3 loss results in alterations in chromatin architecture. Silencing SETD2 impairs the recruitment of the Facilitates Chromatin Transcription (FACT) complex to transcribed chromatin, which results in increased sensitivity to MNase digestion, suggesting alteration in nucleosomal interaction with DNA (Carvalho et al. 2013). This effect was particularly evident at internal exons. Chromatin alterations in response to SETD2 silencing was also observed in a kidney cancer cell line (Kanu et al. 2015). A similar observation was made by examining chromatin accessibility in H3K36me3-deficient primary renal cell carcinomas using formaldehyde-assisted isolation of regulatory elements (FAIRE) (Simon et al. 2014). Overall, enhanced accessibility corresponded to regions typically marked by H3K36me3. By examining individual genic features, signal increases were most striking immediately preceding exons, suggestive of a specific effect at splice acceptor sites. Together,

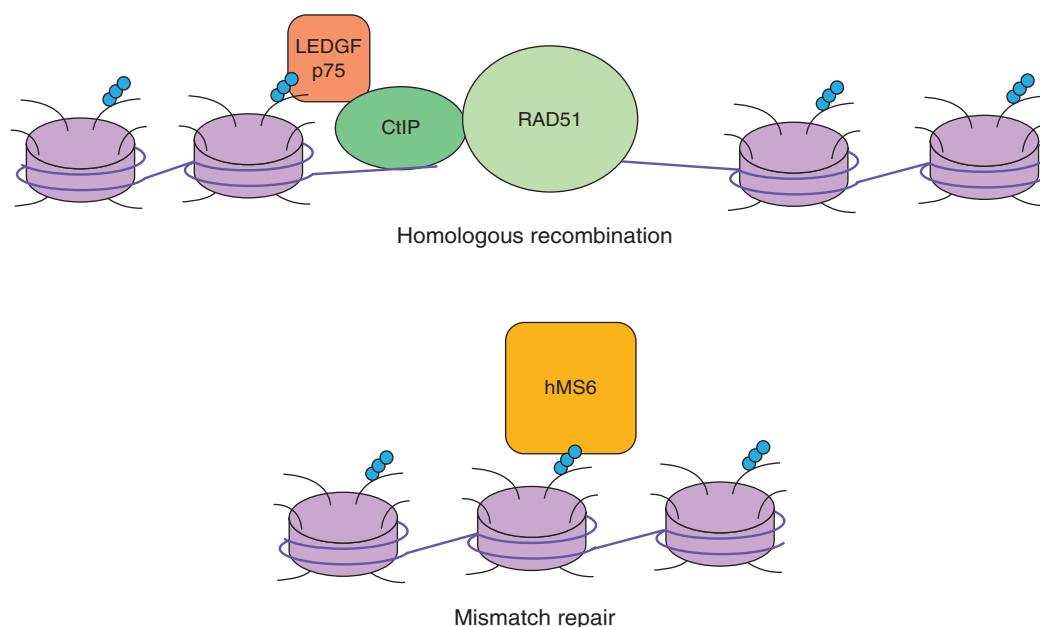
these studies support a link between SETD2, chromatin accessibility and splicing.

### DNA REPLICATION AND DAMAGE REPAIR

Substantial evidence supports the involvement of SETD2 and H3K36 methylation in DNA damage repair by homologous recombination (HR) (Fig. 4). SETD2 silencing resulted in a loss of HR at experimentally induced sites of double-strand breaks (DSBs) (Aymard et al. 2014). In particular, SETD2 loss decreased levels of ATM phosphorylation and consequently p53 phosphorylation (Carvalho et al. 2014) with decreased levels of p53 transcriptional targets (Xie et al. 2008; Carvalho et al. 2014). SETD2 has been shown to associate with and potentially stabilize p53, which may partially account for the difference in transcriptional targets (Xie et al. 2008). SETD2 loss was also associated with decreased recruitment of the HR proteins 53BP1, RPA, and RAD51 to chromatin (Aymard et al. 2014; Pfister et al. 2014; Kanu et al. 2015). This effect seems to be mediated by the histone methylation activity of SETD2 since reintroduction of a catalytically dead SETD2 mutant failed to rescue RPA or RAD51 foci formation, and depletion of H3K36me3 by overexpression of the KDM4 demethylase or H3.3K36M also delayed RAD51 foci formation (Pfister et al. 2014).

LEDGF may bridge H3K36 methylation and the DNA damage response mechanism. In contrast to the role of the p52 isoform in transcription, the LEDGF/p75 isoform recruits carboxy-terminal binding protein and interacting protein (CtIP) to sites of DNA damage (Daugaard et al. 2012). CtIP processes DNA ends to enable binding of RAD51 (reviewed in Symington 2010). Depletion of SETD2 results in decreased LEDGF bound to chromatin, reduced CtIP recruitment to DSB and levels of single-stranded DNA near the DSB, suggesting impaired resection (Pfister et al. 2014). This suggests a model in which regions marked by H3K36me3 by SETD2 are bound by LEDGF following DSB, leading to recruitment of CtIP and RAD51 and, ultimately, repair by HR. Whether SETD2 is recruited to sites of DNA damage that will





**Figure 4.** DNA damage repair. Specific H3K36me3 readers direct either homologous recombination (*top*) or mismatch repair (*bottom*).

go on to repair by homologous recombination, or conversely, that homologous recombination is more likely at regions already marked by H3K36me3 remains unclear. In contrast to H3K36me3, H3K36me2 is rapidly induced after irradiation (Fnu et al. 2011). Increased levels of early nonhomologous end joining (NHEJ) factors were detected by immunoprecipitation of H3K36me2 following radiation.

In addition to HR, SETD2 is involved in mismatch repair (MMR) (Fig. 4). hMSH6, a component of the hMutS $\alpha$  complex that recognizes mismatches in the genome, also contains a PWWP domain that mediates interaction with methylated H3K36 (Li et al. 2013). SETD2 silencing or overexpression of KDM4 decreased MSH6 foci formation associated with increased microsatellite instability (MSI) (Li et al. 2013; Awwad and Ayoub 2015). However, ccRCC tumors samples with biallelic SETD2 loss did not show classic findings of MSI, increased breakpoints or a substantially increased mutation load, compared with tumor cells with monoallelic loss (Kanu et al. 2015). DNA breaks identified in tumors with monoallelic SETD2

loss showed significantly lower levels of H3K36me3. These data are consistent with the model that H3K36me3 marked sites are protected from breakage. Taken together, these studies suggest that H3K36 methylation functions in DNA damage repair with methylation status biasing toward different repair pathways, with dimethylation favoring NHEJ and trimethylation favoring HR and MMR. Although SETD2 deficient kidney cancers are not characterized by increased mutational level, it remains possible that intratumoral heterogeneity limits our ability to detect this feature (Gerlinger et al. 2012).

#### DNA METHYLATION AND REPLICATION

Alterations in DNA methylation have been linked to SETD2 and H3K36me3 loss. The DNA methyltransferases DNMT3A and DNMT3B contain a PWWP domain enabling binding to methylated H3K36 (Dhayalan et al. 2010; Baubec et al. 2015). DNMT3B was enriched at H3K36me3 marked gene bodies, and SETD2 knockout reduced DNMT3B binding (Baubec



et al. 2015). In this study, SETD2 loss was associated with decreased de novo DNA methylation, although a separate study did not observe this association (Hahn et al. 2011). Alterations in DNA methylation correlated with SETD2 loss have been shown in ccRCC. In the TCGA analysis, *SETD2* mutation was associated with decreased DNA methylation at regions that are normally marked by H3K36me3 in kidney (The Cancer Genome Atlas 2013a). Increased chromatin accessibility was also associated with regions of DNA hypomethylation in *SETD2* mutant tumors (Simon et al. 2014). SETD2 loss has also been associated with increased DNA methylation at intergenic regions (Tiedemann et al. 2016). Overall, these data suggest that H3K36me3 directs DNA methyltransferases to gene bodies but in the absence of this histone modification, methylation increases elsewhere.

H3K36me3 levels are cell-cycle regulated with a peak in early S phase then declining to low levels that persist during G<sub>2</sub>/M (Li et al. 2013). This pattern suggests that SETD2 is most active during DNA replication. In support of a role during replication, depletion of SETD2 in kidney cancer cells slowed replication fork progression and led to an accumulation of cells in S phase (Kanu et al. 2015). However, in isogenic SETD2 knockout cell lines, cell-cycle differences were not observed (Pfister et al. 2015).

### SETD2 IN CANCER DEVELOPMENT AND THERAPEUTICS

How SETD2 loss results in cancer development remains unknown. However, several studies have linked H3K36 methylation to aberrant differentiation or proliferation. SETD2 loss disrupts murine embryonic stem-cell differentiation, possibly by altering intracellular signaling (Zhang et al. 2014). Expression of H3.3 mutants that inhibit H3K36 methylation in chondrocytes and mesenchymal progenitor cells disrupted differentiation (Fang et al. 2016; Lu et al. 2016). Mouse mesenchymal progenitor cells (MPCs) that stably express either wild-type or K36M mutant H3.3 formed tumors after subcutaneous injection in immunocompro-

mised mice (Lu et al. 2016). In renal primary epithelial tubule cells, cells considered to be the progenitor for ccRCC, SETD2 knockdown resulted in continued proliferation well past the point at which these cells typically senesce (Li et al. 2016). In models of MLL-rearranged leukemia, SETD2 loss is associated with increased colony formation, proliferation, and accelerated leukemia development after transplantation (Zhu et al. 2014). Taken together, these studies support a role of SETD2 in facilitating faithful differentiation. Interestingly, germline mutations in SETD2 as well as NSD1 (the H3K36 dimethylase; see Fig. 1) have been associated with Sotos and Sotos-like overgrowth syndromes (Kurotaki et al. 2002; Luscan et al. 2014; Tlemsani et al. 2016). Sotos syndrome has been associated with an increased frequency of malignancy, particularly acute leukemias and lymphomas, Wilms tumor, and neuroblastoma (reviewed in Lapunzina and Cohen 2005).

The clinical implications of *SETD2* loss in cancer have primarily focused on ccRCC. *SETD2* mutation is associated with worse cancer-specific survival in the TCGA dataset (Hakimi et al. 2013b). Additionally, *SETD2* mutation was a univariate predictor of time to recurrence, and was found at higher percentages in late stage tumors. Tumors with *BAP1*, *SETD2*, or *KDM5C* mutation were more likely to present with advanced stage (Hakimi et al. 2013a). In metastatic RCC, low SETD2 expression was associated with reduced overall and progression-free survival, and was an independent prognostic marker for these endpoints (Wang et al. 2016). SETD2 expression was lower in breast tumors, compared with matched normal tissue (Al Sarakbi et al. 2009; Newbold and Mokbel 2010), with expression inversely correlated with increasing tumor stage (Al Sarakbi et al. 2009). In these patients, SETD2 mRNA levels were lower in patients with poor outcomes, such as metastasis, local recurrence, and cancer-specific death.

Several studies have explored whether SETD2 loss sensitizes tumor cells to targeted agents. TGX221, a selective PI3K $\beta$  inhibitor, was selectively toxic to RCC cells that were mutant for both *VHL* and *SETD2*, whereas cells



lacking either mutation were not sensitive (Feng et al. 2015). Treatment with this compound resulted in decreased migration and invasion of mutant cell lines. Using a synthetic lethality screening strategy, H3K36me3-deficient cell lines were found to be sensitive to WEE1 inhibition (Pfister et al. 2015). The proposed target of this synthetic lethal interaction is RRM2, a ribonucleotide reductase subunit. SETD2 deficiency and WEE1 inhibition each decreased RRM2 levels, and the combination resulted in further depletion. WEE1 inhibition in the context of SETD2 deficiency critically reduces the dNTP pool causing cells to accumulate in non-replicating S phase, replication stress, and cell death.

### CONCLUDING REMARKS

Large sequencing studies have increasingly implicated mutations in epigenetic modifiers as critical events in cancer development and have identified *SETD2* loss as a key feature of multiple types of cancer. SETD2 has been implicated in many chromatin-directed nuclear processes, including transcriptional regulation, DNA damage repair, DNA methylation, and replication. These effects are likely mediated by H3K36me3-binding reader proteins. Consequently, shifts in H3K36 di- and trimethylation are expected to lead to loss of appropriate reader targeting or redistribution. The relative importance of the H3K36-associated functions to cancer development remains unclear. Although the focus of SETD2 research has been primarily on histone regulation, it is possible that SETD2 may have important nonhistone targets.

As SETD2 mutation is associated with more aggressive cancer, it is important to fully understand the effect of SETD2 loss on oncogenesis. Vulnerabilities created by SETD2 through deregulated transcription and DNA replication may offer therapeutic strategies.

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