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REVIEW

A drive in SUVs: From development to disease

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ABSTRACT
Progression of cells through distinct phases of the cell cycle, and transition into out-of-cycling states, such as terminal differentiation and senescence, is accompanied by specific patterns of gene expression. These cell fate decisions are mediated not only by distinct transcription factors, but also chromatin modifiers that establish heritable epigenetic patterns. Lysine methyltransferases (KMTs) that mediate methylation marks on histone and non-histone proteins are now recognized as important regulators of gene expression in cycling and non-cycling cells. Among these, the SUV39 sub-family of KMTs, which includes SUV39H1, SUV39H2, G9a, GLP, SETDB1, and SETDB2, play a prominent role. In this review, we discuss their biochemical properties, sub-cellular localization and function in cell cycle, differentiation programs, and cellular senescence. We also discuss their aberrant expression in cancers, which exhibit de-regulation of cell cycle and differentiation.

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Abbreviations: AML, Acute myeloid leukemia; Ankrd 1, Ankyrin repeat domain containing protein 1; APC/C-cdh1, Anaphase protein complex/cyclosome-CDC20 homolog 1 protein; BRAF, B-Raf proto oncogene; CBP, CREB-binding protein; cdk2, Cyclin dependent kinase 2; Cdk4, Cyclin-dependent kinase 4; CENP, Centromere protein; DHFR, Dihydrofolate reductase; DNMT1, DNA methyltransferase 1; DOT1L, DOT1 like protein; EHMT1, Euchromatin histone lysine methyltransferase 1; E2F, E2 factor; ERMS, Embryonal rhabdomyosarcoma; EZH, Enhancer of zeste homolog; GLP, G9a like protein; HIV, Human immunodeficiency virus; hMLH1, human MutL homolog 1; HOXA9, Homeobox A9; HP1, Heterochromatin protein 1; IL6, Interleukin 6; KMT, Lysine(K) methyltransferase; mAM/Ham, mouse ATFa-associated modulator /human ATFa-associated modulator; MDM2, Mouse double minute 2 homolog; MEF2, Myocyte enhancer factor-2; MLL, Mixed lineage leukemia; MyoD, Myogenic differentiation; ORC1, Origin recognition complex subunit 1; PCAF, P300/CBP-associated factor; PCNA, Proliferating cell nuclear antigen; Pin1, PeptidyL-prolyl cis-trans isomerase NIMA-interacting 1; PML-NB, Promyelocytic leukemia-nuclear bodies; PRDM, PR/SET domain 1; PUMA, p53 upregulated modulator of apoptosis; Rb1, Retinoblastoma protein 1; SAHF, Senescence associated heterochromatic foci; SASP, Senescent associated secretory phenotype; SET, Su(var)3–9, enhancer of zeste, trithorax; SET1and2, Su(var)3–9,enhancer of zeste, trithorax protein 1and2; SETDB1and2, SET domain bifurcated 1and2; SIRT1, Sirtuin1; SMYD, SET and MYND domain-containing proteins; Ser, Serine; SUV39H1,H2, Suppressor of variegation 3–9 homolog1,2; TNAP-Cre, Tissue non-specific alkaline phosphatase - Cre recombinase; UHRF1, Ubiquitin-like containing PHD and RING finger domain 1; YY1, Yin Yang-1

Introduction

Chromatin modifications are mainly achieved through post-translational modifications (PTMs) such as phosphorylation, sumoylation, acetylation, and methylation of histone tails.[1] Histone phosphorylation on serine, threonine, and tyrosine residues by various protein kinases signal for recruitment of chromatin modifiers leading to either chromatin relaxation or compaction. Similarly, sumoylation of histones causes changes in chromatin and influences gene expression and genome integrity. Histone acetylation causes relaxation of chromatin and gene activation, whereas histone methylation can mark chromatin either to activate or repress gene expression, depending on the lysine residue modified and the degree of methylation (mono-, di-, and tri-methylation). In general, methylation of histone H3 lysine 9 (H3K9) and H3K27 is associated with transcriptional repression, whereas H3K4 and H3K36 methylation is associated with transcriptional activation. Thus, PTMs have myriad consequences on several biologic processes from embryonic development to disease. Lysine methylation mediated by histone lysine methyltransferases (HKMTs) enzymes has emerged as a critical PTM of histones.[2,3] As increasing evidence suggests that these enzymes target non-histone proteins as well, they have been renamed as lysine methyltransferases (KMTs).

With the exception of DOT1L (KMT4) family, which methylates K79, most KMTs contain an evolutionarily conserved SET domain and are referred to as SET domain family proteins.[4,5] The conserved SET domain was first characterized in 3 *Drosophila melanogaster* proteins: Suppressor of variegation 3–9 (Su(var)3–9), Enhancer of zeste (E(z)), and Trithorax (Trx).[4] Based on the sequence homology in and around SET domain, KMTs are further classified into various sub-families, including SUV39, EZH, SET1, SET2, PRDM, and SMYD.[6-8] Each of these subfamilies are comprised of several KMTs. Since

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the discovery of the first KMT, there has been a surge in understanding their function and specificities under physiologic and pathological conditions. In this review, we focus on SUV39 sub-family of KMTs, which includes SUV39H1 (KMT1A),[9] SUV39H2 (KMT1B),[10] G9a (EHMT2/KMT1C),[11] G9a-like protein 1; GLP (EHMT1/KMT1D),[12] SETDB1 (KMT1E),[13] and SETDB2 (KMT1F).[14] We discuss their function in regulation of cellular proliferation, differentiation, and senescence (Fig. 1).

Biochemical properties

SUV39 KMTs mediate methylation by transferring a methyl group from S-adenosyl-L-methionine onto ε amino group on lysine residues in target proteins. They show high specificity for the lysine residue on the substrate, and the degree of methylation defines distinct functional domains of chromatin such as euchromatin and heterochromatin. SUV39H1 and SUV39H2 catalyze H3K9 dimethylation (me2) and trimethylation (me3) by preferentially binding to monomethylated (me1) H3K9.[15,16] G9a and GLP are responsible for the majority of H3K9me1 and H3K9me2 in euchromatin.[12,15] SETDB1 mediates H3K9me1, H3K9me2, and, in association with mAM/hAM—an ATFa associated factor, is able to convert H3K9me2 to H3K9me3 in both euchromatin and heterochromatin.[8,17] SETDB2 also mediates H3K9me3.[14]

SUV39 KMTs also have the ability to read methylation marks. SUV39H1 and SUV39H2 have a Chromo domain, which helps in recognition of methylated lysines.[18] G9a and GLP possess Ankyrin domain required for protein-protein interaction and recognition of H3K9me1 and H3K9me2 marks.[19] The SET domain of SETDB1 and SETDB2 is interrupted by a 347 amino acid insertion, which is conserved from human to lower eukaryotes. The insertion does not affect their catalytic activity.[13,20] SETDB1 and SETDB2 possess a methyl-CpG binding domain that binds to methylated DNA,[21] and, in addition, SETDB1 contains a Tudor domain that helps in methyl-lysine binding.[22] A subset of these KMTs (G9a, GLP, SETDB1, and SUV39H1) have been shown to co-exist in the same complex. Moreover, the stability of KMTs is interlinked as SUV39H1 and G9a null cells show destabilization of all other KMTs.[23]

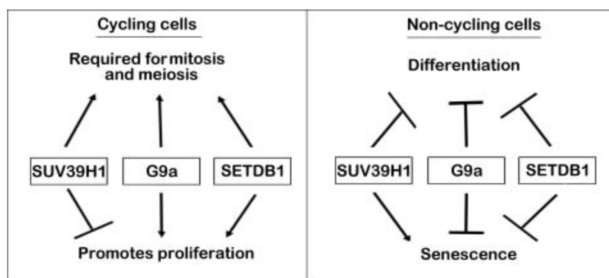

Figure 1. SUV39 KMTs in cycling and non-cycling cells. In cycling cells, SUV39H1, G9a, and SETDB1 are required for chromosomal segregation and cell division during mitosis and meiosis. While SUV39H1 inhibits proliferation, G9a and SETDB1 promote proliferation (G1/S transition) of cells. In non-cycling states, all 3 KMTs inhibit myogenic and adipogenic differentiation but differentially regulate senescence.

Sub-cellular localization during cell cycle

While SUV39H1 protein levels do not vary during different phases of the cell cycle, its distribution is regulated at mitosis. A weak nuclear staining is observed in interphase to early prophase cells. At metaphase, SUV39H1 is concentrated at centromeres that are significantly reduced during anaphase and telophase.[24] The concentration of SUV39H enzymes at centromeres results in heterochromatin compaction that is important for chromosome segregation during mitosis. Posttranslational modifications of SUV39H1 could play a role in its dynamic distribution during mitosis. Indeed, SUV39H1 is phosphorylated at several serine residues at mitosis—potentially at serine (S) 36, S72, S268, S376, S381, S391, and S406, which correlate with centromeric accumulation at metaphase.[24] Cdk2-mediated phosphorylation of SUV39H1 at S391 during S phase leads to its dissociation from chromatin and causes heterochromatin replication.[25] The dynamic distribution during mitosis implies that SUV39H1 may play a role during cell division that has indeed been confirmed by gene disruption studies in mice.

G9a and GLP form homo-and hetero-dimeric complexes and can exert independent functions, although the heterodimeric complex is thought to be functional *in vivo*.[12,26] Similar to SUV39H1, G9a remains nuclear at all phases of the cell cycle, but oscillates with a peak in protein expression at S and M phase.[27] G9a localizes at replication forks through interactions with PCNA and DNMT1. This results in increased binding of DNMT1 to the replication fork and helps in reinstating heterochromatinization through histone and DNA methylation.[28-30]

Interestingly, unlike SUV39H1 and G9a, SETDB1 shows both nuclear and cytoplasmic localization. Within the nucleus, SETDB1 exhibits diffuse staining in euchromatin as well as punctate staining corresponding to PML-NB foci.[31] Moreover, overexpressed SETDB1 is localized mainly in the cytoplasm in various human cell lines, including HeLa, HEK293, HepG2, and A549 cells.[32] Several mechanisms modulate SETDB1 localization. Among these, canonical Wnt signaling is involved in SETDB1 export during muscle differentiation and concomitantly release its occupancy on target genes such as *Ankyrin repeat domain 1* (*Ankrd1*), whose expression is crucial for myogenic differentiation.[33] During the S phase, SETDB1 is localized to pericentric heterochromatin suggesting its function in heterochromatic H3K9me3 during replication.[34]

Developmental functions

SUV39H1 and SUV39H2 show overlapping expression during embryonic development suggesting functional redundancy between these enzymes.[10] Single gene disruption of *SUV39H1* or *SUV39H2* does not affect viability or fertility in mice.[35] However, knockout of both *SUV39H1/H2* results in pre-natal lethality with a substantial reduction in global H3K9me3 levels. The embryos develop normally until E12.5, but later show growth retardation. Loss of SUV39H1/H2 in mice leads to delayed meiotic prophase, and spermatocytes undergo apoptosis at the pachytene stage. Moreover, defects in pericentric heterochromatin lead to genome instability and *SUV39H1* knockout mice show an increased risk of late onset B-cell lymphomas resembling non-Hodgkin lymphomas in humans. A significant

reduction in H3K9 methylation leading to altered gene expression and chromosomal mis-segregation was evident in these lymphomas.[35]

G9a is expressed in several tissues including thymus, skeletal muscle, lymph node, and fetal liver during development.[36,37] Loss of both G9a and GLP in mice leads to embryonic lethality at E9.5 with a global reduction in H3K9me1 and H3K9me2. The embryos show severe growth retardation due to increased apoptosis.[11,12,15] The role of G9a in cell division is apparent by conditional deletion of *G9a* in the germ-lineage using TNAP-Cre, which leads to sterility and aborted meiosis at pachytene stage in germ cells. In addition, a drastic loss in H3K9me1 and H3K9me2 was apparent during meiotic prophase.[38] Similar studies indicate that knockdown of *GLP* in mouse zygotes induces apoptosis and reduced blastocyst formation.[39]

Conditional knockout of *G9a/GLP* in forebrain leads to defect in learning and memory in mice.[40] Deletion of *GLP* (chromosome 9q) have been linked to mental retardation and brachycephaly.[41] Consistently, *GLP* heterozygous mice show delayed postnatal development with brachycephalic crania, a short nose, and hypotonia, resembling features of Kleefstra syndrome.[42] Genetic evidence using SNP genotyping from meduloblastomas has revealed deletions of *GLP* and *SYMD4* genes implicating that histone lysine methylation (H3K9me) could be an important target in these tumors.[43]

SETDB1, but not SUV39H1 and G9a, is expressed in germinal vesicles and metaphase II arrested oocytes. Immunostaining of mouse zygotes revealed a higher level of SETDB1 expression in the male pronucleus localized at the peri-nucleolar rim.[44] Consistent with its unique and early expression, SETDB1 null embryos show peri-implantation lethality at the blastocyst stage at E4.5 compared with SUV39H1 (non-essential) and G9a/GLP (embryos die around E9.5).[44-46] SETDB1-null blastocysts show defective inner cell mass signifying its role in embryonic stem cell formation. In SETDB1 null oocytes, the transition from prophase to metaphase is delayed, and *in vitro* culture of zygotes with maternal SETDB1 deficiency results in a failure to reach the 2-cell stage. The embryos show defects in chromosome segregation and arrest at G2/M.[46] The physiologic function of SETDB2 is lesser characterized although reduction of SETDB2 results in loss of CENP proteins and delayed mitosis.[14] Overexpression of SETDB1 in the adult forebrain of mice results in an anti-depressant like phenotype and impairment in learning.[47] Interestingly, SETDB1, G9a and GLP expression is elevated in patients having schizophrenia.[48] From these studies it is apparent that de-regulation of SUV39 KMTs is associated with neuropathologies. In addition to this, a vast number of studies have also reported that SUV39 KMTs are de-regulated in cancers, which is discussed below in a separate section.

Function in cellular proliferation

In highly orchestrated events during cell cycle, histones and DNA synthesis occur during the S phase, and chromosome segregation and cell division occur during the mitotic M phase. These 2 critical phases are preceded by growth preparatory and checkpoint control known as Gap1 (G1) and Gap2 (G2), respectively. Chromatin modifications are crucial for appropriate gene expression at each cell cycle phase to maintain genome integrity and appropriate progression. SUV39H1 plays a role in the G1/S transition which is regulated by the Rb1/E2F complex. It is now well established that Rb1 associates with E2F1 and creates a repressive complex on cell cycle genes, which ensures their permanent silencing. Repression by Rb1 is partly achieved through recruitment of specific KMTs.[49-51] SUV39H1 associates with Rb1 and mediates repression of cyclin genes by mediating H3K9me3 on their promoters. Consistently, CyclinE and CyclinA2 activity are elevated in SUV39H1 null fibroblasts.[51] Similarly, during early G1, origin recognition complex (ORC1) large subunit interacts with Rb1 and recruits SUV39H1 to the *CyclinE* promoter to mediate repressive H3K9me3 marks. This provides buffering time for daughter cells to decide whether to proliferate or exit the cell cycle.[52] In G0 and early G1, Rb1-SUV39H1 mediated H3K9 methylation is also apparent on the *DHFR* promoter. As the cells progress toward G1/S, Rb1-SUV39H1 dissociates from E2F1 possibly due to hyper-phosphorylation of Rb1, which is correlated with hyperacetylation at the *DHFR* promoter.[53] Interestingly, erythroblasts cultured *ex vivo* from E12.5 fetal liver of SUV39H1 overexpressing transgenic mice show aberrant cell cycle profiles with lower G0/G1, and higher S phase populations. These erythroblasts escape senescence possibly due to reduced p21 and increased Rb1 and p53 expression.[54]

G9a plays a positive role in regulating proliferation of cells.[55] Gene expression studies using microarrays indicate that G9a targets an array of cell cycle genes.[27,56] G9a overexpressing cells progress faster into the S phase, and, conversely, *G9a* knockdown leads to lower S phase cells compared with controls.[27,57] In cultured myoblasts, G9a promotes proliferation via 2 different mechanisms. First, consistent with its repressor role, G9a mediates H3K9me2 marks at promoters of cell cycle inhibitors such as *p21* and *Rb1* in a methylation-dependent manner.[27] Indeed, G9a is well known to transcriptionally repress the expression of *p21*, in several other cell lines.[27,58-60] In addition, G9a interacts with E2F1 and the acetyltransferase PCAF and actively drives the expression of *CyclinD1* and *DHFR* in a methylation-independent manner. G9a occupancy co-relates with active H3K9ac marks while no changes in H3K9me2 are observed on E2F1 target gene promoters.[27] Consistent with these findings, G9a overexpressing myoblasts have increased and sustained CyclinD1 levels.[61] Interestingly, G9a preferentially associates with transcription factor E2F1 during G1/S phase.[27] Evidence indicating higher acetylation at the *DHFR* promoter during G1/S[53] is consistent with the interaction of G9a with the E2F1/PCAF complex during this phase. Human G9a, unlike its mouse counterpart, is also associated with p300/CBP leading to acetylation of *PUMA* promoter that results in apoptosis.[62,63] These studies highlight the methyltransferase dependent and independent function of G9a.

Similar to G9a, SETDB1 is required for proliferation of myoblasts. Knockdown of *SETDB1* reduces S phase cells and results in slower growth.[64] Genome wide analysis revealed SETDB1 occupancy correlated with H3K9me3 on target genes. Interestingly, SETDB1 occupancy was also apparent on promoters with active H3K9ac marks that did not correlate with H3K9me3. This indicates that, similar to G9a, SETDB1 could also be involved in mediating gene activation.[33] In contrast to these observations, in P19 cells, SETDB1 represses the *E2F1*

promoter upon thyroid hormone treatment. Depletion of SETDB1 led to increased colony formation and cells in the S phase. This study indicates a negative role for SETDB1 in proliferation during thyroid hormone receptor regulated transcription.[65]

Function in cellular differentiation and senescence

Cell fate decisions are taken during G1 phase of the cell cycle. In response to distinct stimuli, cells exit the cell cycle and enter into non-cycling states such as differentiation, senescence, and quiescence. The role of SUV39 KMTs has been studied in differentiation of skeletal myoblasts. In muscle cells, differentiation of proliferating myoblasts involves an irreversible arrest that blocks the re-entry of myotubes into the cell cycle. SUV39H1 plays crucial role in silencing of E2F1 target genes in differentiating myotubes by mediating repressive H3K9me3 on *CyclinA* and *DHFR* promoters. In addition, SUV39H1 positions E2F1 target genes close to pericentromeric heterochromatin ensuring silencing of proliferation genes in myotubes. Consistently, knockdown of *SUV39H1* led to inhibition of myogenic differentiation.[66,67] In contrast to these studies, SUV39H1 was shown to inhibit myogenic differentiation by interaction with MyoD and deposition of repressive H3K9 methylation at myogenic promoters.[68] SUV39H1 also functions to inhibit adipogenic differentiation through transcriptional repression of *CEBPα*, a regulator of adipogenesis. Recruitment of both G9a and SUV39H1 by AP2*α* at the *CEBPα* promoter leads to H3K9me2 by G9a, which is further trimethylated by SUV39H1 to inhibit its expression during adipogenic differentiation.[69]

Several groups have addressed the role of G9a and GLP in muscle differentiation using cultured myoblast cell lines as well primary myoblasts.[37,70-74,61] G9a inhibits myogenesis by preventing cell cycle exit through repression of *p21* and *Rb1*[27]. In addition, G9a negatively regulates *MyoD* and *MEF2* transcriptional activity.[70,73,74] Surprisingly, G9a was recently reported to be dispensable for muscle development and regeneration *in vivo*. Satellite cells derived from *G9a* conditional knockout mice (MyoD-Cre) had similar proliferation and myogenic differentiation kinetics compared with controls. Muscle regeneration was also unaffected in *G9a* knockout mice (Pax7-Cre).[75] Given that GLP inhibits myogenesis,[61] and G9a/GLP are thought to function as a heterodimer, it is possible that GLP compensates for G9a loss *in vivo*. In addition, genetic background, which influences mouse phenotypes in many genetically engineered strains, may also account for these findings. Similar to SUV39H1, G9a inhibits adipogenic differentiation.[76-78] This inhibition is mediated via repression of *PPARγ* expression as well as activation of *Wnt10a* expression, a negative regulator of adipogenesis. Deletion of *G9a* or inhibition of its activity with BIX01294, reduced H3K9me2 on *PPARγ* promoter but, interestingly, increased H3K9ac at the *Wnt10a* promoter. Thus, similar to its role in myogenic differentiation, G9a functions in a methyltransferase-dependent and -independent manner to regulate adipogenesis.[77] Furthermore, CEBP*β* activates G9a expression during mitotic clonal expansion reiterating a role for G9a in proliferation of preadipocytes.[78]

In contrast to *G9a*, knockdown of *SETDB1* decreased myogenic differentiation through downregulation of MyoD and myogenin expression indicating that SETDB1 is required for their expression and differentiation. However, surprisingly, overexpression of SETDB1 does not promote differentiation.[64] Another study indicated that SETDB1 promotes the expansion of muscle progenitor cells isolated from muscle fibers and its overexpression inhibits myogenic differentiation. Consistently, similar to *G9a*, knockdown of *SETDB1* at the onset of differentiation led to enhanced differentiation.[33] SETDB1 is also involved in inhibition of adipogenic differentiation. SETDB1 mediates H3K9 methylation on *CEBPα* promoter, and its knockdown led to a decrease in H3K9 methylation and enhanced adipogenic differentiation.[79] Overall, these studies indicate that SUV39 KMTs play a crucial role in cellular differentiation by regulating lineage specific transcription factors (Fig. 2).

Senescent cells exhibit characteristic heterochromatin structures called senescence associated heterochromatin foci (SAHF), which repress expression of proliferation genes.[80] SUV39H1, along with HP1 and Rb1, regulate chromatin changes and silence E2F target genes in senescent cells.[81] Prohibitin, a tumor suppressor gene with anti-proliferative activity interacts with SUV39H1 and HP1 in SAHFs leading to repression of E2F-responsive promoters.[82] Interestingly, SUV39H1 inactivated lymphocytes are refractory to oncogene-induced senescence.[83] Consistently, in Myc driven mouse lymphomas, genetic deletion of *SUV39H1* accelerates tumor development by inactivating cellular senescence.[84] G9a and GLP have also been implicated senescence. Proteosomal degradation of G9a and GLP by APC/Ccdh1, an ubiquitin ligase, was observed during Ras-induced senescence. This led to an apparent decrease in H3K9me2 globally as well as on promoters of *IL-6* and *IL-8*, major components of senescent associated secretory phenotype (SASP), suggesting that G9a-GLP plays a role in senescence.[85] G9a and GLP also play a role in cellular quiescence. E2F6, a repressor member of E2F family, which lacks Rb1 family binding domain, complexes with G9a and GLP in quiescent G0 cells, and silences E2F and Myc responsive promoters.[86] G9a mediated H3K9me2 on HIV1 long-terminal repeat promoter has been implicated in establishment of latent HIV 1 provirus.[87]

De-regulation in cancer

Consistent with its role in suppressing genes required for proliferation, SUV39H1 plays a tumor suppressor role. Mutations in Rb1 disrupt its interaction with SUV39H1 in many cancers.[51] In a zebrafish model for embryonal rhabdomyosarcoma (ERMS), SUV39H1 exerts its tumor suppressive effect by reducing tumor initiation. SUV39H1 overexpression resulted in senescence and growth arrest of ERMS cells by silencing the expression of *CyclinB1*, an E2F1 target gene.[88]

SUV39H1 activity is regulated by PTMs that impinge on its function in cell cycle progression and tumorigenesis. For instance, SUV39H1 stability is negatively regulated by Pin1 and

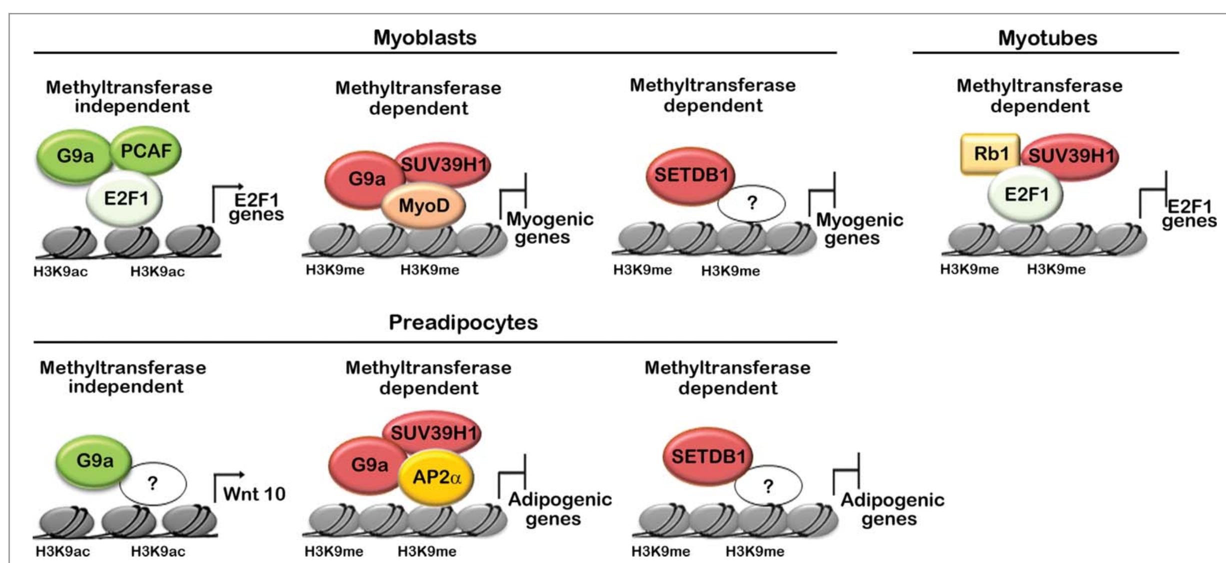

Figure 2. Regulation of cellular differentiation by SUV39 KMTs. G9a associates with both activator as well as repressor complexes in proliferating myoblasts and pre-adipocytes. SUV39H1 and SETDB1 are mainly involved in repression of myogenic and adipogenic genes. In differentiating myotubes SUV39H1 represses E2F1 target genes and ensures permanent cell cycle exit. The transcription factors that recruit SETDB1 in myoblasts and pre-adipocytes and G9a in pre-adipocytes have yet to be identified.

positively by SIRT1. Ser16 phosphorylation of Pin1 leads to its association with SUV39H1 and marks it for ubiquitin mediated degradation. Pin1-dependent degradation of SUV39H1 promotes carcinogenesis via upregulation of CyclinD1 which under normal conditions is repressed by SUV39H1. Upregulation of CyclinD1 results in increased proliferation and tumorigenesis.[89] SIRT1 increases SUV39H1 half-life by inhibiting its polyubiquitination at K87 by MDM2, and thus maintains genome stability.[90] Upon DNA damage, SUV39H1 is methylated at K105 and K123 by SET 7/9 methyltransferase. This leads to a decrease in its methyltransferase activity and a drop in H3K9me3 leading to heterochromatin relaxation and genomic instability in cancer cells.[91] On the other hand, deacetylation of lysine 266 by SIRT1 increases SUV39H1 activity resulting in increased H3K9me3 at heterochromatin regions.[92]

In most instances, G9a regulates cell cycle either by suppression of tumor suppressors or activating oncogenes. Not surprisingly therefore, G9a and GLP are upregulated in various human cancers.[93-96] G9a is significantly increased in stage 4 neuroblastomas. Inhibition of G9a results in decreased CyclinD1, Cdk4, and Cdk6 expression causing G1 arrest.[97] Similarly, high expression of G9a in colorectal cancer is associated with tumor progression and maintenance of malignancy. Silencing of *G9a* in colorectal cancer cells is associated with increased DNA damage resulting in the upregulation of senescence markers, such as p21 and senescence-associated β-galactosidase.[98] In mouse models of acute myeloid leukemia, loss of G9a alters proliferation and delays disease progression. G9a loss in AML leads to G0/G1 arrest, which is partially mediated by HoxA9 dependent gene expression.[99] In contrast to its potential oncogenic role, some lines of evidence indicate it may function as a tumor suppressor. G9a is an upstream regulator of Ubiquitin like with PHD and Ring Finger Domains 1 (UHRF1), a master regulator that controls the expression of many tumor suppressors, such as $p16^{INK4A}$, hMLH1 (mutL homolog), p21, and Rb1 in various cancers. G9a, along with the co-repressor YY1 was found to silence the expression of UHRF1. Downregulation of UHRF1 led to a delay in S phase progression, thereby preventing tumor progression in leukemic cells.[57] Thus, the effects of G9a may be cancer specific.

The role of SETDB1 in tumorigenesis is less understood. p53 seems to play an important role in most of the effects of SETDB1 in tumorigenesis. SETDB1 is overexpressed in human prostate cancer cells and is associated with tumor progression. Silencing of SETDB1 led to a decrease in proliferation primarily due to a cell cycle arrest at the G1 phase.[100] Increasing reports indicate a critical role for SETDB1 in liver carcinogenesis.[101,102] In liver cancer cells, *p53* undergoes a gain of function mutation (R249S), and also show an overexpression of SETDB1. SETDB1 methylates *p53* at lysine 370 and, thereby, stabilizes it to regulate cell growth. Attenuation of SETDB1 abrogates the methylation of mutant *p53*, resulting in a decrease in S phase cells and proliferation.[102] Paclitaxel, a chemotherapeutic drug, negatively regulates SETDB1 expression. The repressive action of paclitaxel on SETDB1 is mediated by p53-dependent recruitment of SUV39H1 at the *SETDB1* promoter resulting in increased H3K9 repressive marks.[103] In glioma cell lines and tissue samples, SETDB1 and SUV39H1 expression is elevated. Inhibition of SETDB1 and SUV39H1 independently led to a decrease in proliferation as a result of increased apoptosis.[104]

Future perspectives

SUV39 KMTs have emerged as critical determinants of cell cycle progression and cell fate decisions (Table 1). As discussed above, aberrant expression of KMTs is apparent in cancer and, although not a focus of this review, in neurologic disorders.[105,106] Since KMTs are reliant on S-adenosyl methionine as a cofactor, their role in linking metabolism to tumorigenesis is an interesting avenue for further investigation. Epigenetic modifications are selective and reversible. As such, KMTs are being intensively investigated as drug targets. Indeed, ongoing efforts

Table 1. A summary of the roles of SUV39 KMTs in cell cycle progression, differentiation, senescence, and their de-regulation role in cancer.

	CELL CYCLE PROGRESSION	DIFFERENTIATION	SENESCENCE	CANCER
SUV39H1/H2	Loss of SUV39H1/H2 delays meiotic prophase in spermatocytes.[34]	Inhibits myogenic differentiation by repressing MyoD activity[68]	Promotes senescence by repressing E2F1-target genes.[81]	Functions as a tumor suppressor. Knockout results in increased B-cell lymphomas[34]
	Inhibits proliferation by repressing E2F1 target genes.[51-53]	Inhibits adipogenic differentiation by repressing C/EBPα expression.[69]		Overexpression in ERMS leads to delay in tumor initiation.[88]
G9a/GLP	Loss of G9a in germ cells aborted meiosis at pachytene stage.[38] Promotes proliferation by inducing expression of E2F1 target genes. Prevents cell cycle exit through repression of p21 and Rb1[27].	Inhibits myogenic differentiation by repressing MEF2 and MyoD activity.[70,73,74] Inhibits adipogenic differentiation by repressing PPARγ and activating Wnt10a expression.[77]	Inhibits senescence by repressing IL6 and IL8 expression.[85]	Overexpressed in several cancers including breast, lung ovarian and colorectal cancer[93-96] Loss leads to delay in disease progression in acute myeloid leukemia.[61]
SETDB1/B2	SETDB1 null oocytes show delayed transition from prophase to metaphase.[46] Promotes expansion of muscle progenitor cells.[33] Knockdown slows growth in myoblasts.[64]	Overexpression inhibits muscle differentiation[46] Inhibits adipogenesis by repressing CEBPα.[79]	Prevents BRAF induced oncogenic senescence in melanoma.[110]	Overexpressed in prostrate and liver cancer[100-102]

toward understanding biochemical and structural properties of KMTs have led to the development of highly selective small molecule inhibitors that target enzymatic activity. In exciting advances, DOT1L and EZH2 inhibitors have entered clinical trials for AML and MLL respectively.[107] It is, however, important to note that recent studies have identified methyltransferase activity independent functions of KMTs.[27,62,108] In addition, although each SUV39 KMT is known to regulate gene expression and exert independent functions, a subset (G9a, GLP, SETDB1, and SUV39H1) have been shown to co-exist in the same complex, and their stability is interlinked.[23] Thus multiple KMTs may function synergistically in human pathologies. However, the inhibition of both H3K9 and H3K27 methylation may not be desirable as it can lead to age related pathologies.[109] Thus, a broader understanding of their mechanisms of action and regulatory circuits is essential to develop targeted therapies in human pathologies.

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