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Review Article

SET and MYND domain containing protein 3 in cancer

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Abstract: Lysine methylation plays a vital role in histone modification. Deregulations of lysine methyltransferases and demethylases have been frequently observed in human cancers. The SET and MYND domain containing protein 3 (SMYD3) is a novel histone lysine methyltransferase and it functions by regulating chromatin during the development of myocardial and skeletal muscle. It has been recently unveiled to play significant roles in human cancer genesis and progression via regulating various key cancer-associated genes and pathways and promoting cell proliferation and migration. Upregulation of SMYD3 expression is present in multiple cancer types, suggesting it as a potential prognostic marker. Herein the structure, substrates and targets of SMYD3, and its effects on initiation, invasion and metastasis of diverse tumors (*e.g.*, esophageal squamous cell carcinoma, gastric cancer, hepatocellular carcinoma, cholangiocarcinoma, breast cancer, prostate cancer, and leukemia) are systematically reviewed, providing clues for the development of novel SMYD3-specific personalized anti-cancer therapy. SMYD3 inhibitors (*e.g.*, BCI-121 and novobiocin) could hopefully fight against tumors with efficacy.

Keywords: SMYD3, cancer, histone lysine methylation, tumorigenesis, tumor progression, targeted therapy

Introduction

Cancer incidence remains rising worldwide, presenting a significant health burden nowadays [1]. Tumor biology is now believed to be regulated by classical genetic mechanisms, as well as epigenetics [2]. Histone methylation, a vital epigenetic mechanism, is mediated by histone methyltransferases and demethylases. It plays key roles in alteration of chromatin structure, resulting in the regulation of DNA replication and gene expression. In human carcinogenesis, deregulation of the histone modification process is a crucial step in transcriptional regulation. As lysine methylation signaling deregulation is a common etiological factor in tumorigenesis, inhibitors of several histone lysine methyltransferases (HKMTs) have been developed as therapeutics [3]. The SET and MYND domain containing proteins (SMYDs) constitute a unique family of the multi-domain SET-containing HKMTs and a new class of chromatin regulators, which are important in myocardial and skeletal muscle development and in tissue injury response [4, 5]. The SMYD homologs exist in diverse organisms, and are also implicated in human cancers [6]. For SMYD2 and SMYD3, the methylation activity has been clearly described [7, 8]. SMYD3 is especially focused on in cancer research due to its essential role in tumor cell growth and its increased expression in various cancer types, particularly those driven by the Ras signaling activation [9, 10]. The majorly cytoplasmic SMYD3 was initially found to be a novel SET domain-containing histone H3-lysine (K) 4-specific N-lysine di- and tri-methyltransferase [10]. However, it is recently revealed that SMYD3 has no virtual activity on H3 in a nucleosome context; rather, it methylates histone H4 at K5, a non-canonical site, which seems to be far more effective than H3 [11]. It ectopically combines with RNA polymerase II to form a transcriptional complex [10]. In early embryonic lineage commitment, SMYD3 plays an important role through the activation of lineage-specific genes. Likewise, its frequent upregulation and overexpression are associated with genesis and progression of multiple human tumors [8]. Interestingly, whole-body or organ-specific SMYD3

deletion dose not generate obvious pathological alterations in mice [8]. The complexity of SMYD3 biology and its great promise in targeted drug development make it a hot topic recently.

SMYD3 structure, substrates and targets in oncology

SMYD3 participates in the formation of the active site of chromatin, and directly binds to the regulatory regions of target genes, regulating the transcription [12, 13]. It offers the first example that other regions besides the SET domain and its flanking regions are implicated in chromatin and tumor regulation. Structural analysis of the full-length human SMYD3 in complex with S-adenosyl-L-homocysteine shows a tetratrico-peptide repeat (TPR) domain, together with which the SET and post-SET form a deep, narrow substrate binding pocket, which constitutes the C-terminal domain (CTD) of SMYD3. Both TPR and post-SET domains are critical for the HKMT activity of SMYD3, and the hydroxyl group of Tyr239 plays vital roles in the enzymatic activity. The characteristic MYND domain is located close to the substrate binding pocket, exhibiting a largely positively charged surface. The DNA binding of SMYD3 may be mediated by the MYND domain via direct DNA binding, leading to the stimulation of its HKMT activity [14]. The crystal structure analysis of full-length SMYD3 in complex with the methyltransferase inhibitor sinefungin reveals that SMYD3 possesses a 2-lobed structure with the substrate binding cleft located at the bottom of a 15-A-deep crevice formed between the N- and C-terminal lobes. Comparison of SMYD3 and SMD1 clearly indicates that the CTD can undergo a large hinge-bending motion that defines distinct conformations, and SMYD3 partially blocks the substrate binding cleft via a closed conformation with the CTD. Therefore the potentiation of the proteins requires posttranslational activation, *e.g.*, through molecular chaperon heat shock protein (HSP) 90 [15].

It is implicated that the SMYD3 histone methyltransferase (HMTase) and the nuclear chaperone, HSP90, which regulate the cellular SMYD3 localization in normal and cancer cells, are independent proto-oncogenes in several human malignancies. A degenerate TPR-like domain encoded in the SMYD3 CTD regulates the physical interaction with HSP90 [16, 17]. The CTD of SMYD3 plays a key role for its basal HMTase activity, while the TPR-like structure is essential for HSP90-enhanced enzyme activity. Loss of SMYD3-HSP90 interaction leads to SMYD3 mis-localization within the nucleus, resulting in reduction of SMYD3-mediated cell proliferation by the loss of its chromatin association. Potentially this procedure could cause impairment of SMYD3 oncogenic activity [17]. Human cancer cells express SMYD3 protein of both the full-length and a cleaved form. Amino acid sequence analysis uncovers that the cleaved form of SMYD3 protein lacks the 34 amino acids in the N-terminal region of the full-length form. Interestingly, compared to the full-length protein, the cleaved and mutant ones show a higher HMTase activity because of 2 highly conserved amino acids, glycines 15 and 17, in the N-terminal region. Furthermore, the N-terminal region connects with HSP90α, whose presence enhances histone H3K4-specific methyltransferase activity in the SET domain of SMYD3. Therefore, the N-terminal region is essential for the modulation of methyltransferase activity, which may consist of a structural change of the protein through the cleavage of the region or interaction with HSP90α [18]. These suggest that a novel therapeutic for blocking HSP90-driven malignancies in SMYD3-overexpressing cells may emerge to reduce toxicity profile over current HSP90 inhibitors.

The methylation substrates of SMYD3 contain histones (*e.g.*, H3K4 and H4K5), vascular endothelial growth factor receptor (VEGFR) 1, MAP3 kinase (MAP3K) 2, *etc.* [19]. The crystal structure of the full length human SMYD3 in a complex with an analog of the S-adenosyl methionine (SAM) methyl donor cofactor reveals an overall compact composition. In this architecture the 'split-SET' domain employs a canonical SET domain fold and closely assembles with a Zn-binding MYND domain and a C-terminal super-helical 9 α-helical bundle, and such procedure is similar to SMYD1 structuring in mouse. These structurally interlocked domains form a highly confined binding pocket for histone substrates. It is previously undetected that the unique structural elements, both inside and outside the core SET domain, play regulatory roles in tri-methylation of H4K20 via mutational and biochemical analyses [20]. VEGFR1 and MAP3K2 peptides are the co-crystal struc-

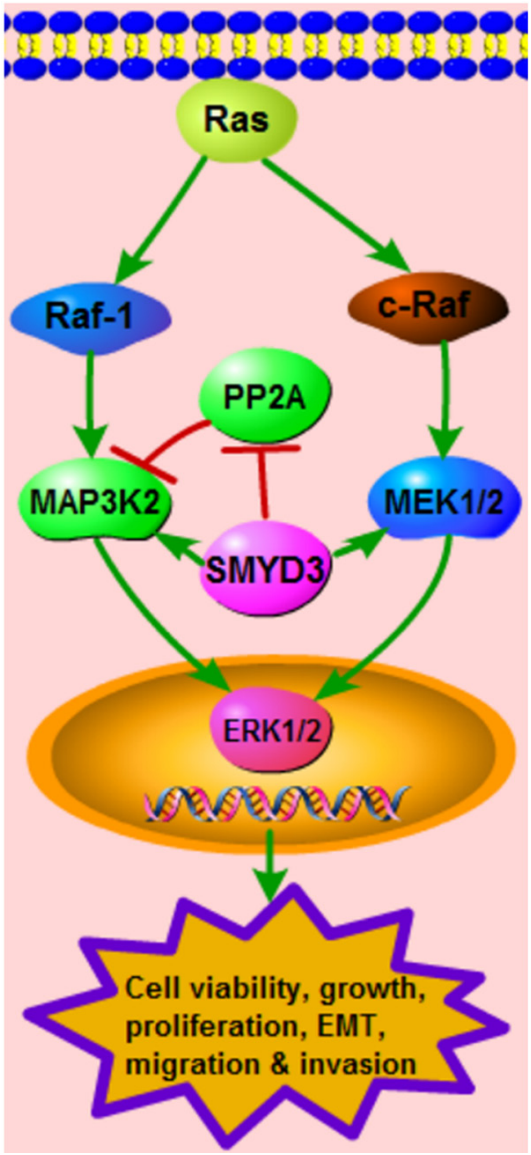

Figure 1. SMYD3 as a new enhancer in Ras-driven cancers via its cytoplasmic functions. Mutant *Ras*-induced activation of ERK1/2 is promoted by SMYD3-mediated methylation of the cytoplasmic kinase MAP3K2, which impedes its binding to the phosphatase PP2A, leading to the up-regulated activity of the canonical RAS pathway. Thereby the impact of phosphatase PP2A, a negative regulator of Ras-ERK1/2 signaling, is inhibited, resulting in the formation of various adenocarcinomas. The PP2A phosphatase complex binds to MAP3K2, attenuating the MAPK pathway. Its methylation impacts this interaction. Activation of the MAPK and Ras/Raf/MEK/ERK signaling module is potentiated by SMYD3-mediated methylation of MAP3K2. SMYD3, SET and MYND domain containing protein 3; MAPK, MAP kinase; EMT, epithelial-mesenchymal transition.

tures of SMYD3, due to the presence of a phenylalanine residue at the -2 position. Structural and biochemical analyses confirm that MAP3K2 serves as a robust substrate of SMYD3. A shallow hydrophobic pocket on SMYD3 provides the binding region of the phenylalanine, promoting efficient catalytic activities of SMYD3. By contrast, SMYD3 displays a weak activity toward a VEGFR1 peptide. Drastic conformational rearrangements for juxtaposition of the acceptor lysine with the enzymatic active site is required for the location of the acceptor lysine in the folded kinase domain of VEGFR1 [21]. It is confirmed further that the methylated residue at VEGFR1 lysine 831 is located in the kinase domain, conserved among VEGFR1 orthologues. Serine, which is conserved among some of the methylation targets of HMTases, follows lysine. In cells the kinase activity of VEGFR1 is enhanced by its methylation [19]. These findings have contributed towards the profound understanding of the biological role of SMYD3 and regulatory mechanisms of VEGFR1, especially the cytoplasmic portion, which may additionally promote the development of strategies through inhibiting the growth of tumor cells.

SMYD3 trans-activates the telomerase reverse transcriptase (*hTERT*) gene directly, which is vital for cellular transformation and immortalization. SMYD3 occupies its binding motifs on the *hTERT* promoter and contributes to inducible and constitutive hTERT expression in both normal and malignant human cells, owing to the maintenance of histone H3K4 tri-methylation. It is suggested that SMYD3-mediated trimethylation of H3K4 functions as a licensing element for subsequent transcription factor binding to the hTERT promoter, and that the hTERT gene is a direct target of SMYD3 which mediates cellular transformation. Knocking down SMYD3 in tumor cells abolishes tri-methylation of H3K4, weakens the occupancy by the trans-activators c-MYC and Sp1, and causes diminished histone H3 acetylation in the *hTERT* promoter region, followed by down-regulation of hTERT mRNA and telomerase activity [22]. Histone H4 methylation is a novel histone methylation mark detected in diverse cell types. It is catalyzed by SMYD3 at lysine 5, and depletion of SMYD3 protein attenuates its formation. In addition, the enzymatic activity of histone H4 is essential regarding SMYD3-driven cancer cell phenotypes. Thus, SMYD3, via H4K5 methylation, provides a potential new connection between chromatin dynamics and

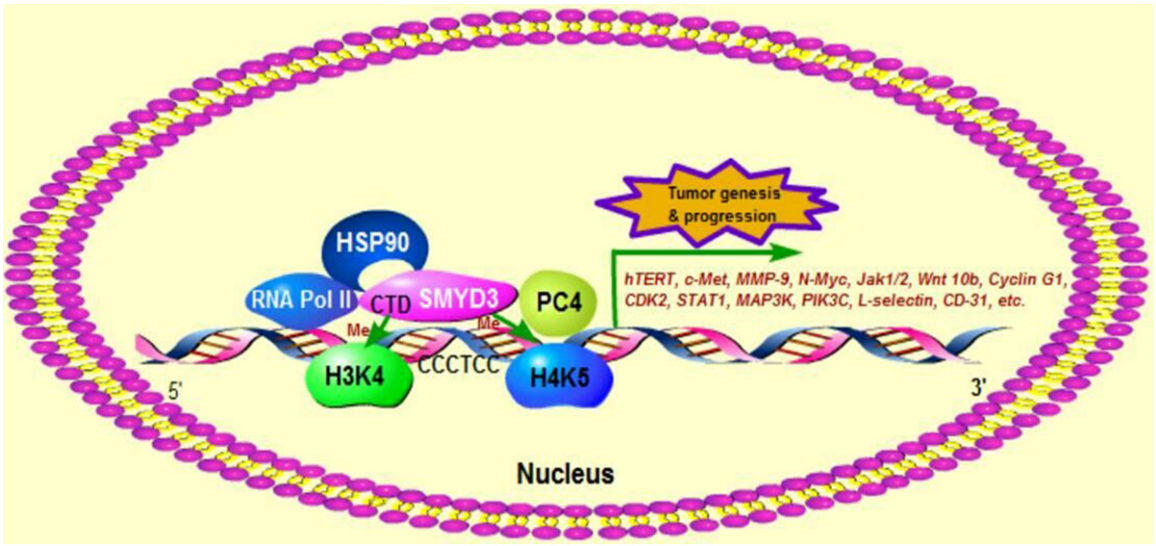

Figure 2. Nucleic functions of SMYD3 via its methylation activity in cancer cells. When SMYD3 is localized in the tumor cell nucleus, which is potentially mediated by HSP90, it serves as a transcription activator promoting various genes through forming a complex with RNA Pol II and PC4, and binding to specific DNA sequences (5'-CCCTCC-3'), where H3K4 and H4K5 are methylated, further modulating other histone modifications and chromatin remodeling. SMYD3, SET and MYND containing protein 3; HSP90, heat shock protein 90; RNA Pol II, RNA polymerase II; CTD, C-terminal domain; Me, methylation.

neoplastic diseases [11]. These should be taken into consideration to explore novel inhibitors targeting SMYD3 methyltransferase activity when fighting against genesis, progression, and metastasis of MAP3K2 and *Ras*-driven cancers.

SMYD3 and tumor genesis and progression

Epigenetic control of gene expression is essential in the development and progression of various tumor types. As a gene-specific transcriptional regulator, SMYD3 has been implicated in cell growth and cancer pathogenesis, influencing distinct oncogenic processes. Lysine methylation has been traditionally related to histones and epigenetics. Recently, it has been observed that lysine methyltransferases and demethylases are deregulated frequently in human tumors. In this respect, SMYD3 has been unveiled as a novel enhancer of *Ras*-driven tumor (**Figure 1**). SMYD3 is up-regulated in different types of cancers. Mutant *K-Ras*-induced activation of ERK1/2 is increased by SMYD3-mediated methylation of the cytoplasmic kinase MAP3K2 (MEKK2), which is markedly more potent than the effect on histones, and which impedes its binding to the phosphatase PP2A, leading to the up-regulated activity of the canonical RAS pathway [8, 23]. Thereby the impact of phosphatase PP2A, a negative regulator on Ras-ERK1/2 signaling, is prevented, resulting in the formation of various adenocarcinomas. Besides, depletion of SMYD3 synergizes with a MEK inhibitor which blocks *Ras*-driven pancreatic neoplasia. These underscore the importance of lysine methylation in the regulation of signaling pathways relevant to tumorigenesis. Drugs targeting unregulated lysine methylation are endorsed as therapeutic agents against cancer [24]. In the formation of RAS-driven carcinomas, the MAP kinase (MAPK) signaling is elevated by methylation of MAP3K2 via SMYD3. In mouse models for pancreatic ductal and lung adenocarcinomas, tumor development in response to oncogenic Ras is inhibited by abrogating SMYD3 catalytic activity. In cancer cell lines, activation of the Ras/Raf/MEK/ERK signaling module is potentiated by SMYD3-mediated methylation of MAP3K2, and Ras-driven tumorigenesis is blocked by a MEK inhibitor, which is synergized by SMYD3 depletion. The PP2A phosphatase complex binds to MAP3K2, attenuating the MAPK pathway. Its methylation impacts this interaction [8]. The expression profiling (*i.e.*, occurrence of metastasis and persistent disease, and disease-related death) significantly rises in the more aggressive diseases with *EZH2* and *SMYD3* gene expression. The up-regulations of

EZH2 and SMYD3 do not associate significantly with mutational statuses of *RET* or *RAS* genes. Thus, the HMTase EZH2 and SMYD3 mRNA expression may represent useful prognostic biomarkers and tailor the most appropriate follow-up and timing of therapeutic approaches [25].

SMYD3 also exerts pivotal nuclear functions (**Figure 2**). Through interacting with the human positive coactivator (PC) 4, SMYD3 potentiates a group of genes whose expression is linked to cell proliferation and invasion. As SMYD3 cooperates functionally with PC4, the depletion of PC4 leads to the loss of SMYD3-mediated trimethylation of histone H3K4 (H3K4me3) and target gene expression. It is indicated that SMYD3 and PC4 localize at target genes in a mutually dependent manner. Depleting SMYD3 and PC4 individually results in diminishing the recruitment of both SMYD3 and PC4. SMYD3 mutant is incapable of binding to its cognate elements and interacting with PC4 on target genes. It is sufficient to artificially tether a SMYD3 mutant with PC4 for achieving an active transcriptional state in SMYD3-deficient cells. Thus, by stabilizing SMYD3 occupancy at target genes, PC4 contributes to SMYD3-mediated transactivation primarily and they collaborate to stimulate oncogenic transcription [26].

Over-expression of SMYD3 plays an important role in cell viability, adhesion, invasion, and migration. Knock-down of SMYD3 in tumor cells significantly reduces the biological function of HGF and inhibits migration and invasion of cancer cells. The high-affinity receptor of HGF is encoded by the proto-oncogene *c-Met*, and the HGF-c-Met signaling pathway is essential for carcinogenesis. Overexpression of SMYD3 is accompanied with high c-Met expression, suggesting a partial correlation between SMYD3 and c-Met. Transcription of *c-Met* gene is down-regulated by silencing SMYD3 in tumor cells via specific shRNAs, on the contrary, overexpressing SMYD3 promotes c-Met transcription. There are 2 SMYD3 binding sites within the *c-Met* core promoter region, which are significant in the transactivation of *c-Met* [27]. Upregulation of the matrix metalloproteinase (MMP)-9 stimulates cell migration, tumor invasion, and angiogenesis, and plays a critical role in tumor progression and metastasis [28]. In a reversible model of cancer initiated by infection with intracellular Theileria parasites, gene induction by parasite infection correlated with H3K4me3 at the MMP-9 promoter. In particular, SMYD3 is the only HMTase upregulated upon infection. Overexpression of SMYD3 is obtained in many types of cancer cells and contributes to malignant pathophysiology. SMYD3 overexpression induces the expression of MMP-9 in transformed leukocytes and fibrosarcoma cells, which is further enhanced by pro-inflammatory phorbol esters. Thus, SMYD3 is capable of increasing cell migration through MMP-9 expression. In contrast, when SMYD3 is knocked down by RNA interference, H3K4me3 modification of the MMP-9 promoter decreases, which suppresses MMP-9 expression, and leads to reduction of tumor cell proliferation. Furthermore, SMYD3 ablation also diminishes cellular invasion in a zebrafish xenograft model of cancer. Consequently, SMYD3 is an important new regulator of MMP-9 transcription, providing a molecular link between SMYD3 overexpression and cancer metastatic progression [9].

SMYD3 plays a crucial role in transcriptional regulation of carcinogenesis and development of human cancers. SMYD3 specifically activates the transcription of a set of downstream genes, including several oncogenes (*e.g.*, *N-Myc*, *CrkL*, *Wnt10b*, *RIZ*, and *hTERT*), cell cycle genes (*e.g.*, *Cyclin G1* and *CDK2*), signal transduction genes (*e.g.*, *STAT1*, *MAP3K11*, and *PIK3CB*), and cell adhesion genes (including *N-Myc*, *CrkL*, *Wnt10b*, *L-selectin*, *CD31*, and *galectin-4*). These genes have been shown to have effects on cell viability, adhesion, and migration. After SMYD3 expresses in mouse fibroblast NIH3T3 cells, several transformed phenotypes are shown by foci formation and colony growth in soft agar. Besides, these transfectants also demonstrate increased dependence in serum, depressing the sensitivity to dexamethasone-induced apoptosis [29]. In NIH3T3 cells stably transfected with the human *SMYD3* gene, there is an elevated proliferation rate in the transfected cells, which becomes more resistant to dexamethasone-induced cell death. Furthermore, the SMYD3-transfected cells also exhibits increased rates of cell adhesion to both type IV collagen and endothelial cells, leading to enhanced cell migration ability in both 2- and 3-dimensional assays. Thus, as associated with cell adhesion and migration in pathological processes, SMYD3 might be a potential novel and promis-

Table 1. SMYD3-associated genes in various cancers

Cancer type	SMYD3-associated genes
Esophageal squamous cell carcinoma	*EZR, LOXL2, RIZ1*
Gastric cancer	*TGF-β1, STAT3/pSTAT3, MMP-9*
Hepatocellular carcinoma	*C-MYC, RIZ1*
Cholangiocarcinoma	*RASSF1A, DNMT1, MIR124, C-MYC, MMP9*
Breast cancer	*ERα, WNT10B, MRTF-A, MYL9*
Prostate cancer	*CCND2, AR*
Glioma	*p53*
Bladder cancer	*BCLAF1*
Leukemia	*SUV39H1, Tax*

SMYD3, SET and MYND domain containing protein 3.

ing target of therapeutic intervention for cancer treatment [30]. Introduction of SMYD3 into NIH3T3 cells promotes cell growth, while genetically knocking down SMYD3 with small-interfering RNAs (siRNAs) in cancer cells causes significant growth suppression. SMYD3 interacts with the RNA helicase HELZ and forms a complex with RNA polymerase, resulting in the trans-activation of oncogenes, homeobox genes, and cell-cycle genes. SMYD3 presents in the promoter region of downstream genes (*e.g.*, *Nkx2.8*) by binding to the motif 5'-CCCTCC-3' [10]. These suggest that, as a member of an RNA polymerase, SMYD3 plays an important role in transcriptional regulation. Activation of SMYD3 may be a key factor in the genesis and development of human cancers [10]. Overexpressed SMYD3 is observed in various cancers, *e.g.*, colorectal and hepatocellular carcinomas, the leading causes of cancer deaths in some regions of the world. In mouse model, cancer formation is induced chemically by SMYD3 expression. This progress includes transcription of cancer-promoting genes, such as *Jak1/2*, *Myc*, and *Ctnnb1*, which are promoted by SMYD3 in the nucleus. Nuclear SMYD3 functions in stimulating the transcription of several key regulators, which are involved in cell proliferation, epithelial-mesenchymal transition (EMT), the JAK/Stat3 oncogenic pathway, as well as the *Myc* and *Ctnnb1* oncogenes. Moreover SMYD3 interacts with H3K4me3-modified histone tails, whose recruitment target the core promoter regions of the most active genes. The binding density of SMYD3 on target genes positively associates with increased RNA polymerase-II density and transcriptional outputs. Although the function of SMYD3 is widespread distributed, its transcription-potentiating action is restricted to a specific group of genes, whose expression is facilitated particularly during carcinogenesis [31].

SMYD3 in different cancer types

The expression of SMYD3 is increased in various tumors (**Table 1**). Upregulation of SMYD3 induced carcinogenesis through significant associations exist between homozygosity with a 3 tandem repeats sequence of a CCGCC unit in the regulatory region of SMYD3, thus elevating the risk of colorectal cancer (odds ratio [OR] = 2.58), hepatocellular carcinoma (OR = 3.50), breast cancer (OR = 4.48), *etc.* This tandem-repeat allele is a binding site for the transcriptional factor E2F-1. It is implied that the common variable number of tandem repeats polymorphism in SMYD3 is a susceptibility factor for human cancers, as plasmids containing 3 repeats of the binding motif (equivalent to the high-risk allele) have stronger activities than those containing 2 repeats (the low-risk allele) from a reporter assay [32].

Esophageal squamous cell carcinoma (ESCC)

There have been multifactorial elements involved in the etiology of ESCC such as genetic, epigenetic, and environmental factors, especially tobacco smoking. A variable number of tandem repeats (VNTR) polymorphism has demonstrated to be functional in the promoter region of *SMYD3*, which is implicated in cell proliferation and carcinogenesis. Wang *et al.* [33] genotyped 567 patients with newly diagnosed ESCC and equal number of healthy controls to explore the association between VNTR and ESCC risk. The result uncovered a significant association between increased ESCC incidence (OR = 1.42) and the common SMYD3 VNTR genotype. Stratification analysis unveiled that the increased risk was significantly restricted to smokers (OR = 1.99). Moreover, in comparison with the non-smokers carrying the homozygous or heterozygous genotypes, ORs of the wild genotype for non-smokers, smokers

who smoked < 25, and ≥ 25 pack-years were 1.03, 2.80, and 4.76, respectively. It suggested a correlation between this genetic polymorphism and smoking status. These findings support that the common VNTR polymorphism in the promoter region of SMYD3 gene, interacting with tobacco carcinogens, may represent a susceptibility factor for human cancers such as ESCC.

The regulation of ESCC initiation and progression consists of epigenetic alterations, including DNA methylation and histone modifications. SMYD3 plays an important role in regulating transcription transcriptional regulation during human tumor formation. Patients with low SMYD3-expressed tumors have much longer overall survival (OS) compared with those carrying high SMYD3-expressed cancers. Overexpression of SMYD3 is significantly related to lymph node metastasis in ESCC, suggesting that itis a significantly independent negative prognosticator. Knockdown of SMYD3 inhibits ESCC cell proliferation, migration and invasion *in vitro*, likewise suppresses local cancer invasion *in vivo*. SMYD3 binds to the sequences of the promoter regions of *EZR* and *LOXL2* directly, regulating the transcription of these target genes. There is a significantly positive correlation between SMYD3 expression and the protein levels of EZR and LOXL2. Thus, through enhancing transcription of genes involved in proliferation, migration, and invasion, SMYD3 stimulates tumourigenicity in ESCC [34]. In paired tissue samples, SMYD3 protein expression is dramatically higher in the cancer tissues (72.7%) than in the normal ones (18.2%). The mRNA expression level of SMYD3 is diminished mostly by Plasmid SMYD3-shRNA-1242. In TE13 cells the over-expression of the anti-oncogene *RIZ1* is promoted by Downregulation of the SMYD3 mRNA and protein expression levels. Ablation of SMYD3 expression significantly suppresses the proliferation of TE13 cells. As overexpression of SMYD3 correlates with occurrence of ESCC and its suppression inhibits cancer cell proliferation, SMYD3 may participate in the biological activity of ESCC. RNAi downregulation of SMYD3 induces RIZ1 expression in TE13 cells, suggesting a signal transduction pathway between SMYD3 and RIZ1. This signaling pathway may provide a novel therapeutic target for ESCC [35].

Gastric cancer (GC)

SMYD3 plays an important role in the development of human gastric carcinoma (GC). In GC cell lines MKN28, SGC7901, and MGC803, the expression levels of SMYD3 mRNA and protein are higher than that in normal gastric mucosa cell line GES-1. Aslo SMYD3 expression in GC is significantly associated with primary tumor size, lymph node metastasis, and TNM stage, and identified as the significantly independent negative prognostic factor of the OS in overall GC patients (hazard ratio [HR] = 0.564) as well as in patients with positive lymph node metastasis (HR = 0.529). besides SMYD3 is essential in the GC aggressiveness and may function as a promising target for prognostic prediction [36]. It is confirmed that the mRNA and protein expression levels of SMYD3 and Transforming growth factor (TGF)-β1 in GC tissues are much higher than those in adjacent non-tumor tissues. There exists a significantly positive relation between SMYD3 and TGF-β1 expressions in GC tissues [37]. Furthermore, the mRNA expression levels of SMYD3 or STAT3 and the protein expression levels of SMYD3, STAT3/pSTAT3 in GC tissues are significantly higher than those in adjacent non-tumor tissues. And SMYD3 and STAT3/pSTAT3 expressions in GC tissues are significantly positively correlated. They are suggested to be independent GC prognosticators, with high expressions potentially indicating poor prognosis of GC patients [38]. SMYD3 and MMP-9, which is correlated to tumor progression, are overexpressed in human cancers. Both mRNA and protein expression levels of SMYD3 and MMP-9 in GC are significantly higher than those in adjacent non-tumor tissues. There is a positive association between SMYD3 and MMP-9 expressions in GC tissues and they act as the independent predictor in GC. Therefore, SMYD3 and MMP-9 may also play critical roles in tumor invasion, metastasis, and prognosis and could serve as promising targets for prognosticator for GC [39].

Hepatocellular carcinoma (HCC)

SMYD3 is over-expressed in HCC, having a procarcinogenic effect for carcinogenesis. SMYD3 mRNA and protein and C-MYC protein are significantly higher in HepG2.2.15 compared with that in HepG2. In HepG2 cells, SMYD3 and

C-MYC expressions are enhanced by HBX transfection, leading to decreased cell apoptosis and increased cell proliferation. Knocking down SMYD3 in HBX transfected HepG2 impacts C-MYC expression and promotes apoptosis. These elucidate that SMYD3 expression is upregulated by HBX in HepG2, promoting hepatoma development and progression. C-MYC may serve as a down-stream gene in HBX-SMYD3-related hepatocarcinogenesis [40]. SMYD3 overexpression in HCC is involved with RIZ1 hyper-methylation and mRNA down-expression. *RIZ1* CpG promoter is significantly de-methylated by suppression of SMYD3 expression, which increasing RIZ1 mRNA expression. Consequently, SMYD3 down-expression with RIZ1 de-methylation distinctively inhibits the growth and migration of hepatoma cell, also induces apoptosis in HepG2 cells. All these information confirms that SMYD3 plays a vital l role in the carcinogenesis and progression of HCC The proliferation, migration induction and apoptosis inhibition activities of SMYD3 may be achieved by hyper-methylation of RIZ1 CpG promoter [41].

Cholangiocarcinoma

There is increasing evidence indicating the importance of epigenetic regulation in cholangiocarcinoma genesis. The transfection of hepatitis C virus core (HCVc) protein, a major protein for intrahepatic cholangiocarcinoma development, makes normal biliary cells transforming to malignant ones. And in many hilar cholangiocarcinoma patients, tumor suppressor gene *RASSF1A* is downregulated by hypermethylation in the promoter region. SMYD3 is overexpressed in patients with cholangiocarcinoma especially in those with HCV infection. In hilar cholangiocarcinoma cell lines QBC939 and FRH0201, the expression of SMYD3 could be upregulated when Transfecting HCVc into cells, resulting in promotion of cell growth. This phenomenon is consistent with the conclusions s of the clinical research, indicating that SMYD3 is correlated to the epigenetic regulation of cholangiocarcinoma genesis with HCV infection. Furthermore Overexpression of SMYD3 could inhibit RASSF1A expression, aberrantly inhibition of SMYD3 improves its expression. SMYD3 regulates PASSF1A promoter by its methylation status. Thus, HCVc could upregulate the methylation status of the RASSF1A promoter through regulation of SMYD3, and in this way the DNA methylation of downstream gene is affected by histone methylation [42]. MicroRNAs (miRNAs, miRs) are another important group of regulators contributing to tumor progression by interacting with downstream target genes. It is observed that miR-124 is down-regulated in HCV-cholangiocarcinoma, mediating through the induction of DNMT1 by HCVc. Over-expression of miR-124 reduces the protein levels of SMYD3 and downstream target genes (*c-Myc* and *MMP9*), suppressing cell migration and invasion *in vitro*. Knockdown of SMYD3 inhibits cell migration and invasion, which resembles that of miR-124 over-expression. Thus, by targeting SMYD3, low levels of miR-124, which is mediated by HCVc via DNMT1, promote cholangiocarcinoma cell migration and invasion [43].

Breast cancer (BC)

The overexpression of SMYD3 is frequently found in various types of BC cells. SMYD3 exerts its function in estrogen receptor (ER)-mediated transcription though HMTase activity, regulating the development and progression of tumor. SMYD3 acts as a coactivator of ERα, which promotes ERα activity in response to ligand. Through interacts directly with the ligand binding domain of ER, SMYD3 is recruited to the proximal promoter regions of ER target genes, potentiating gene induction. Importantly, SMYD3 is required to accumulate di- and tri-methylation of H3K4 at the induced ER target genes. Furthermore, it is suggested that SMYD3 is responsible for ER-regulated gene transcription in estrogen signaling pathway. Therefore, as a new coactivator for ER-mediated transcription, SMYD3 functions as a potential link between ER expression and BC [44].

SMYD3 plays an important role in transcriptional regulation in tumorigenesis of human BC, and HSP90α induced the activity of SMYD3. Upregulation of SMYD3 is observed in a large number of BC tissues, playing a key role in cancer cell proliferation. Similarly to CRC and HCC, silencing of SMYD3 leads to inhibition of BC growth, growth of BC cells, confirmed the importance of SMYD3 expression for the proliferation of BC cells. Specifically, SMYD3 regulates the expression of the proto-oncogene *WNT10B* directly and results in the promotion

of breast carcinogenesis. These findings imply that augmented SMYD3 expression is essential for BC development, and that silencing SMYD3 should be a novel therapeutic strategy for BC treatment [45]. After transfecting with SMYD3, The MDA-MB-231 cells, shows different transformed phenotypes. Down-regulation of SMYD3 could induce G1-phase cell cycle arrest, leading to the potent stimulation of apoptosis. These results describes the regulatory mechanisms of SMYD3 on the acceleration of cell cycle and facilitate the exploration of agents targeting the progression of cell cycle in BC cells [46].

Myocardin-related transcription factor-A (MRTF-A), a Rho signal-responsive transcriptional coactivator of serum response factor (SRF), might play an important role in regulating mammary gland, involving in cancer metastasis. Myosin regulatory light chain 9 (MYL9) is an important cytoskeletal component which is implicated in cell migration. The upregulation of MYL9 is mediated by MRTF-A through histone methylation. Overexpression of SMYD3 induces MRTF-A-mediated upregulation of MYL9 and leads to more migration of MCF-7 BC cells. On the contrary, downregulation of MYLT and inhibition of cell migration are present when the endogenous MRTF-A and SMYD3 are suppressed. Moreover it is suggested by the mutation analysis that, the proximal binding element of MRTF-A in the promoter of *MYL9*, as well as the HMT activity of SMYD3, mediate the above cooperative transactivation. In this regard, MRTF-A together with SMYD3 regulate transcription and cell migration in BC [47].

Prostate cancer (PrC)

PrC is the most frequently diagnosed cancer worldwide in men, however the clinical and pathological parameters are incapable to differentiate adequately between clinically aggressive and indolent PrC. Varied expression of HMTases and histone methylation patterns are present in prostate carcinogenesis. SMYD3 transcript levels are elucidated to have prognostic values by discriminating among PrC with different clinical aggressiveness. Knockdown of SMYD3 causes the attenuation of malignant phenotype of LNCaP and PC3 cell lines, which is similar to the deletions of the SET domain mediated through its HMT activity, regulating the formation of tumor. SMYD3 also regulates a putative target gene CCND2 via tri-methylation of H4K20. These results show that the proto-oncogenic function of SMYD3 in prostate carcinogenesis is established by its methyltransferase enzymatic activity [48]. Androgen receptor (AR) plays a crucial role in PrC formation and is frequently overexpressed during the development and invasion of tumor. There exists an upregulation of SMYD3 protein expression of 88% in prostate tumor specimens, in comparison with matched normal tissues. In PrC tissues, a strong SMYD3 staining is observed by immunohistochemical analysis in 32% of the nuclei and 92% in the cytoplasm, whereas benign prostate tissue shows weak immunostaining. PrC cell proliferation, colony formation, cell migration, invasion, and xenograft tumor formation are significantly suppressed by depleting SMYD3. Moreover, two functional SMYD3-binding motifs are confirmed in the AR promoter region [13]. Therefore SMYD3 promotes prostate tumorigenesis by mediating the epigenetic upregulation of AR expression, suggesting it as a potential predictor for clinically aggressive tumor and an attractive therapeutic target in PrC.

Other solid tumors

The discovery of a great quantity of HMTases unveils important roles of these enzymes in regulating carcinogenesis and tumor progression. In various malignancies, SMYD3 overexpression is a frequent genetic abnormality, activating the transcription of both oncogenes and cell cycle genes. Elevated expression of SMYD3 is found in human glioma but not in normal brain tissue. There is a distinct correlation between the expression levels of SMYD3 protein in human glioma tissues with the tumor grade. Glioma patients' survival is inversely associated with SMYD3 protein level. Enforced SMYD3 expression induced the proliferation of glioma LN-18 cell, while by inhibiting SMYD3 expression in glioma T98G cells, their anchorage-independent growth is suppressed *in vitro* and tumorigenicity *in vivo*. Furthermore, SMYD3 regulates the expression of p53 protein, which plays a key role in SMYD3-promoted cell growth in glioma cells. These indicate that the overexpression of SMYD3 in human glioma contributes to glioma tumorigenicity via p53. Therefore, SMYD3 may provide a new potential therapeutic target for human malignant glioma [49].

There is a strong association between SMYD3 with poor prognosis of bladder cancer (BIC) patients. The upregulation of SMYD3 in BIC is related to progression and poor prognosis of tumor. SMYD3 frequently amplified results in BIC cell proliferation and invasion, whereas SMYD3 ablation suppresses cancer cell growth and invasion. Mechanically, SMYD3 is suggested to stimulate the expression of BCL2-associated transcription factor 1 (BCLAF1). SMYD3 accumulates di- and tri-methylation of H3K4 at the BCLAF1 locus and interacts with promoter of *BCLAF1*, leading to the upregulation of *BCLAF1*. In B1C cells, SMYD3 overexpression induces autophagy activation, while BCLAF1 depletion attenuating SMYD3-promoted autophagy. Thus SMYD3 enhances BIC progression, at least in part by upregulation of BCLAF1 expression and autophagy activation. These findings establish a function for SMYD3 in autophagy activation and BIC progression, providing a candidacy as a new prognosticator and target for therapeutic for of BIC [50].

In the SMYD3-silenced HeLa cells induced by doxycycline, SMYD3 mRNA and protein expressions are both diminished, leading to reduction of cell proliferation, colony formation and migration/invasion activity. After knocking down SMYD3, The amount of cells in sub-G1 is increased and DNA ladder formation could be detected, suggesting that SMYD3 functions as potent induction of apoptosis. Therefore SMYD3 is essential in HeLa cell proliferation and migration/invasion, and it may be a potential useful therapeutic target in human cervical carcinomas [51].

Leukemia

SMYD2 and SMYD3 are the most widely studied and well-characterized in SMYD family. Their varied expression is suggested to be correlated with the progression of several solid tumors. Upregulation of SMYD2 and SMYD3 are observed in chronic lymphocytic leukemia (CLL) patients and, interestingly, CLL patients with residual expression of both genes presents a high WBC count and complex karyotype. In addition, there exists a significant association between SMYD2 and SMYD3 gene expression. A residual expression of SMYD2 and SMYD3, regulated by a common transcriptional control in CLL, is implied to indicate CLL progression. Thus the strategies modifying SMYD2 and SMYD3 could be developed as novel therapeutics to prevent CLL progression [52]. HTLV-1 Tax, mainly mediated by its protein-protein interactions with host cellular factors, deregulates signal transduction pathways, transcription of genes, and cell cycle regulation of host cells. There exists an interaction of Tax with an HMTase, SUV39H1, mediated by the SUV39H1 SET domain and could stimulate gene transcription. The SUV39H1 SET domain is shared among HMTases, as well as SMYD3 methylates histone H3 lysine 4. T cell lines and primary T cells express endogenous SMYD3. And the direct link between Tax and SMYD3 is largely dependent on the C-terminal 180 amino acids of SMYD3, while the N-terminal 108 amino acids are dispensable for the interaction. Tax is co-localized with SMYD3 in the cytoplasm or nuclei in the co-transfected cells. Furthermore, its subcellular localization is dominated by SMYD3. The presence of SMYD3 enhances activation of Nuclear Factor-κB (NF-κB) through cytoplasmic Tax, the contrary result is present when knocking down SMYD3. These demonstrate that SMYD3 upregulates Tax level in the cytoplasm. Hence apparent tethering of Tax by SMYD3 influences the subcellular localization of Tax. Due to the interaction of cellular proteins localized in the cytoplasm or nucleus, SMYD3-mediated nucleocytoplasmic shuttling of Tax is responsible for the pleiotropic effects of Tax, which are mediated by [53].

SMYD3-targeted anti-cancer therapy

SMYD3 participates in transcriptional activation as a member of an RNA polymerase complex, and its oncogenic role has been described in a range of different cancer types. Some SMYD3-inhibitory small molecules have been currently developed via targeting the lysine methyltransferase activity [23, 54, 55]. SMYD3 is strongly upregulated throughout tumorigenesis both at the mRNA and protein level in a preclinical model of colorectal cancer (CRC). SMYD3 ablation impairs the proliferation of CRC cell, which indicates SMYD3 is required for proper cancer cell growth. Lysine methyltransferases is an important target for drug discovery, and a virtual screening to identify new SMYD3 inhibitors was performed by testing several candidate small molecules. One of these compounds (BCI-121) reduced SMYD3 activity strongly both *in vitro* and in CRC cells, as suggested by analyzing global H3K4me2/3

and H4K5me levels. Of note, the extent of cell growth suppression by BCI-121 is similar to that upon SMYD3 knockdown. SMYD3 inhibitors are also effective in other cancer cell lines of different origin, including lung, pancreatic, prostate, and ovarian tumors. These evidences prove that SMYD3 is a drugable target, and new compounds which are capable of inhibiting its activity may be useful as novel therapeutic agents in cancer treatment [56].

Target validation studies identify a novel series of selective small molecule oxindole SMYD3-inhibitors via screening of the Epizyme proprietary HMTase-biased library. Two compounds, sulfonamide EPZ031686 and sulfamide EPZ-030456, are afforded with optimized cellular potency at a level sufficient to probe the *in vitro* biology of SMYD3 inhibition. In mouse model, EPZ031686 shows good bioavailability following oral dosing, suggesting it is a suitable tool for potential *in vivo* target validation researches [54]. Novobiocin is a HSP90 inhibitor and could decrease the expression of SMYD3. It provides dose-dependent inhibition of the proliferation and migration of MDA-MB-231 human BC cells. Similar to novobiocin, the short hairpin RNA, which targets SMYD3 gene, also has such a controlling effect. The suppressive effect of novobiocin on the migration of BC cells is associated with the downregulation of SMYD3. Thus, these findings might lend further understanding to the potential role of SMYD3 in human carcinogenesis, throwing light on the development of novel therapeutic approaches to human cancers [57]. In patients with CRC, mutations of the *KRAS* oncogene are predictive for antibody therapeutic resistance against the epithelial growth factor receptor (EGFR). The introduction of drugs, which inhibit signaling pathways activated by *KRAS* mutations could potentially solve this treatment dilemma. There is a strong correlation between the presence of activating *KRAS* mutations and the upregulation of cell migration-promoting genes (*e.g.* DUSP4, a MAP-kinase phosphatase, and SMYD3). Their expression could be inhibited by the MEK1-inhibitor PD98059 and the antibacterial compound novobiocin. These imply a potential approach to overcome EGFR antibody resistance in patients with *KRAS*-mutant rectal carcinomas [58].

Summary

This review highlights the vital role of lysine methylation in the regulation of transcription factors, tumor-associated proteins, and signaling pathways, through both its nucleic and cytoplasmic functions. The deregulation of lysine methylation leads to carcinogenesis and tumor progression. Based on its molecular structure, SMYD3 is a crucial element in a range of cellular processes, *e.g.* cell viability, growth, proliferation, adhesion, migration, and invasion. It is widely overexpressed in various tumors, and plays a key role in tumor genesis, progression, and metastasis, according to various functional studies in cell lines and animal models. It promotes the transcription of several key regulators, which are involved in cell proliferation, EMT, and oncogenic signaling pathways (*e.g.*, the JAK/Stat3 and the HGF-c-Met pathways). Moreover, SMYD3 forms a complex with RNA polymerase II, results in trans-activating a set of key oncogenes (*e.g.*, *N-Myc* and *hTERT*), homeobox genes, and cell cycle-related genes. It is suggested to be a significant prognosticator for tumor formation, development and metastasis. However, the cross-talk between SMYD3 and the downstream factors still requires further exploration. Besides, the regulatory mechanism of intracellular SMYD3 levels and localization, and of its potentially discrepant oncogenic activities in diverse tumor entities remains obscure. It might exert location-specific functions. The researches identifying SMYD3 functions in cancer provides potential clues for novel and effective lysine methyltransferase-targeted anti-cancer therapeutics [59].

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Disclosure of conflict of interest

None.

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