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Akt methylation by SETDB1 promotes Akt kinase activity and oncogenic functions

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Running title: SETDB1 methylates and activates Akt

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ABSTRACT

Aberrant activation of Akt disturbs proliferation, survival and metabolic homeostasis of various human cancers. Thus, it is critical to understand upstream signaling pathways governing Akt activation. Here, we report that Akt undergoes SETDB1-mediated lysine-methylation to promote its activation, which is antagonized by the Jumonji-family demethylase, KDM4B. Notably, compared with wild-type mice, mice harboring non-methylated mutant *Akt1* not only exhibited reduced body size, but also were less prone to carcinogen-induced skin tumors in part due to reduced Akt activation. Mechanistically, Phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) interaction with Akt facilitates its interaction with SETDB1 for subsequent Akt methylation, which in turn sustains Akt phosphorylation. Pathologically, genetic alterations including *SETDB1* amplification aberrantly promote Akt methylation to facilitate its activation and oncogenic functions. Thus, Akt methylation is an important step synergizing with PI3K signaling to control Akt activation, suggesting that targeting the SETDB1 signaling could be a potential therapeutic strategy for combatting hyperactive Akt-driven cancers.

INTRODUCTION

Epigenetic regulation, such as DNA methylation and histone modifications, plays important roles in governing gene expression patterns during human development and disease progression [1, 2]. Inhibitors targeting epigenetic factors have been explored for cancer therapies and have undergone clinical trials, including DNA methyltransferase 1 (DNMT1) inhibitors, histone deacetylases (HDAC) inhibitors, and histone methyltransferase inhibitors [3-5]. Among these epigenetic inhibitors, histone methyltransferase (such as EZH2 and DOT1L) inhibitors display impressive efficacy in cancer patients [6, 7]. This robust efficacy may also be attributed to regulating methylation of non-histone proteins such as Rb and p53, in addition to regulating histone methylation [8-11]. However, it remains largely unknown whether predominant oncogenic signaling pathways that are frequently activated in human cancers, such as PI3K/Akt signaling pathway, are subjected to methylation-dependent regulation. Thus the identification of the major oncogenic proteins governed by methylation is critical to identify new therapeutic targets.

Hyperactivation of PI3K/Akt signaling is a central module of cell proliferation, survival and metabolic homeostasis in human cancers [12, 13]. Physiologically, stimulations derived from various types of growth factors tend to activate Akt, which in turn phosphorylates distinct substrates to perform different biological processes [13, 14]. Recently, emerging evidence has demonstrated that distinct signals govern Akt kinase activity e.g. TRAF6/Skp2-mediated positive regulation of Akt in an ubiquitination-dependent manner [15], CDK2/Cyclin A-mediated positive regulation of Akt in a tail phosphorylation-dependent manner [16], and pVHL-mediated negative regulation of Akt in a hydroxylation-dependent manner [17]. However, the regulation of Akt as a non-histone substrate by histone methyltransferases is not well defined.

Here, we described that Akt1 methylation in its Linker-region is mediated by the histone methyltransferase SETDB1, which is antagonized by the demethylase KDM4B. Biologically, absence of Akt1 methylation attenuates its kinase activity, represses cell growth, glucose uptake, and tumorigenesis. As a result, deficiency in Akt methylation can physiologically decrease mouse body size and can protect mice from developing carcinogen-induced skin tumors. Thus, our data unravel a profound role for the SETDB1/KDM4B axis in manipulating Akt activity and highlight histone methyltransferase SETDB1 as a potential target for combating hyperactive Akt-driven tumors.

RESULTS

Methylation of Akt enhances its kinase activity

To identify important non-histone proteins regulated in a methylation-dependent manner, we used a specific pan-lysine tri-methylation (K-me3) antibody and performed a mass spectrometry (MS) based screening on cell lysates derived from ovarian cancer cells (Fig. 1a and Supplementary Fig. 1a) [10]. Notably, an Akt1-derived peptide was identified, containing methylated modifications at two nearby evolutionarily conserved lysine residues (K140 and K142) in the Akt1 Linker region (Table S1, Fig. 1b). Furthermore, Akt1 methylation was validated using the K-me3 antibody in cells treated with a global histone methylation inhibitor, 3-Deazaneplanocin A (DZneP) (Supplementary Fig. 1b).

Additional MS analyses on ectopically expressed Akt1 further confirmed the tri-methylation of K140 and K142, where mono- and di-methylation events were also identified (Supplementary Fig. 1c). Moreover, K64, another lysine residue in the PH domain of Akt1, was also identified to be mono-methylated in our system. Interestingly, mutation of both K140 and K142 residues led to a dramatic reduction of Akt1 K-me3 signal in cells (Supplementary Fig. 1d), indicating that K140 and K142 might be the major Akt1 K-me3 residues. In addition, consistent with a previous report [18], deletion of *Smyd3*, a methyltransferase targeting MAP3K2, did not disturb Akt kinase activity in cells, although the interaction of SMYD3 with Akt1 has been previously reported (Supplementary Fig. 1e-g) [19]. Given that both K140 and K142 sites were identified at endogenous levels through our large-scale non-biased MS approach, we chose to mainly focus on understanding the contribution of K140 and K142 tri-methylation to Akt activity and oncogenic functions in the remainder of this study.

Absence of Akt methylation represses its oncogenic functions in cells and increases resistance to carcinogen-induced skin tumors *in vivo*

In order to reveal the potential biological functions of tri-methylation within its Linker region of Akt1, a methylation-deficient variant of Akt1 (K140R and/or K142R) was ectopically expressed in *AKT1* and *AKT2* double knockout DLD1 cells (DLD1-$AKT1/2^{-/-}$). Compared to Akt1-WT, the Akt1-K140/142R mutant, and to a lesser extent, Akt1-K140R or -K142R, exhibited a significantly reduced activation, as shown by a marked reduction in Akt-pT308 and Akt-pS473, as well as its downstream targets, pGSK3β and pFOXO3a (Fig. 1c and Supplementary Fig. 1h-j). This observation is coupled with a reduction of Akt1 kinase activity observed *in vitro* (Supplementary Fig. 1k). As a consequence, absence of methylation on K140 and K142 led to reduced cell colony formation, anchorage-independent growth, glucose uptake, and lactate production *in vitro* (Fig. 1d, e and Supplementary Fig. 1l, m) and tumor growth *in vivo* (Fig. 1f-h and Supplementary Fig. 1n, o).

To explore the role of Akt methylation under physiological conditions, we generated Akt1-K140/142R double mutations in HEK293 cells (termed $AKT1^{K140/142R}$) using CRISPR/Cas9 (Supplementary Fig. 1p-r) [20]. Notably, K140/142R mutation of endogenous Akt1 decreased Akt phosphorylation in response to various stimuli (Fig. 1i and Supplementary Fig. 1s-u), and also compromised cell growth, colony formation, glucose uptake, and lactate production (Fig. 1j-m). Next, we generated Akt1-K140/142R double mutation knock-in mice (termed $Akt1^{KI/KI}$) (Supplementary Fig. 2a-c). Compared with the WT mice, $Akt1^{KI/KI}$ mice exhibited a decrease in body size/weight (Fig. 2a, b and Supplementary Fig. 2d-f), organ size/weight (Fig. 2c and Supplementary Fig. 2g) and Akt kinase activity (Fig. 2d, e and Supplementary Fig. 2h), phenocopying $Akt1^{-/-}$ mice [21]. To pinpoint whether methylation deficient mutants of *Akt1* abrogate tumorigenesis *in vivo*, we utilized a two-step chemical carcinogen

(DMBA followed by TPA)-induced skin tumor model [22]. In this model, we observed that *Akt1$^{KI/KI}$* mice displayed a lower incidence of skin tumors and reduced papilloma burden compared with WT mice (Fig. 2f-h), which correlated with decreased Akt signaling (Fig. 2i). These results together support the notion that methylation of Akt in its Linker region likely promotes Akt kinase activity and oncogenic functions both *in vitro* and *in vivo*.

SETDB1 methylates and activates Akt

Next, we set out to identify the physiological upstream methyltransferase(s) for Akt. Consistent with previous reports [23-25], we found that there is a relatively strong physical interaction between Akt1 and two tri-methyltransferases, SETDB1 and EZH2 (Fig. 3a and Supplementary Fig. 2i). Interestingly, genetic alterations of both *SETDB1* and *EZH2* tended to mutually exclusive of alterations in PI3K/Akt pathway-related genes including *PTEN* deficiencies, *EGFR*, *PIK3CA* or *AKT1* amplification/mutations in human melanoma and breast cancer (Supplementary Fig. 2j) [26]. Furthermore, SETDB1, but not other methyltransferases including EZH2, could enhance Akt tri-methylation in cells (Supplementary Fig. 2i). This result indicates that unlike EZH2 as a downstream substrate of Akt [25], SETDB1 may be an upstream regulator of Akt methylation (Supplementary Fig. 2k).

Notably, SETDB1 specifically interacted with the PH domain of Akt1, and to a lesser extent, Akt2 and Akt3 (Fig. 3b, Supplementary Fig. 2l, m). Interestingly, mutation of Akt1 methylated residues (K140/142R) could markedly decrease the interaction of Akt1 with SETDB1 (Supplementary Fig. 3a). Importantly, the Tudor domain of SETDB1 was identified to bind Akt1, which was enhanced by enforcing expression of WT, but not the SETDB1-H1224K catalytic inactive mutant (Supplementary Fig. 3b, c). These results indicate that SETDB1 could be a potential reader of the Akt1-K140/142 methylation

events through its Tudor domain, and by enhancing its interaction with Akt1, SETDB1 could promote Akt1 methylation on additional residues (such as K64). In addition, SETDB1 promoted Akt1 tri-methylation in an enzymatic activity-dependent manner in cells (Supplementary Fig. 3d), and mutation of either K140 or/and K142 residue could decrease SETDB1-mediated Akt tri-methylation in cells (Supplementary Fig. 3e). Moreover, ^{3}H-SAM-mediated *in vitro* methylation assays also indicate SETDB1 could directly methylate Akt1 at K140 and K142 residues (Fig. 3c). Hence, these results coherently support the notion that SETDB1 serves as a putative Akt1 methyltransferase.

SETDB1 promotes cell growth and glycolysis through methylation of Akt

As histone H3 methyltransferase capable of catalyzing tri-methylation on the lysine 9 of histone H3 (H3K9me3) [27], SETDB1 is amplified in various cancers [28-31]. Notably, we identified that, in addition of being in the nucleus and modifying histone marks, SETDB1 was also observed in the cytoplasm of cells (Supplementary Fig. 3f). To further elucidate the physiological functions attributed to methylated-Akt1, we generated specific antibodies against tri-methylation of Akt1 at K140 (termed K140-me3), which was validated by dot blot and immunoprecipitation assays (Supplementary Fig. 3g, h), and recognized SETDB1-mediated tri-methylation of Akt1 at K140 in cells (Supplementary Fig. 3i, j) and *in vitro* (Supplementary Fig. 3k, l). Additionally, genetic depletion of *SETDB1* markedly reduced Akt1-K140-me3 in cells (Fig. 3d-f and Supplementary Fig. 3m). Compared with Akt1-WT, a cancer patient-derived Akt1-E17K mutant [32] displayed an enhanced interaction with SETDB1, coupled with an increase of its K140 methylation level (Fig. 3g and Supplementary Fig. 3n-o). These findings suggest that Akt1 is a *bona fide* methylation substrate of SETDB1.

Importantly, genetic deletion of *Setdb1* in cells diminished Akt activation (Fig. 3e, f, h and Supplementary Fig. 3p-s). Furthermore, depletion of endogenous *SETDB1* by shRNA also reduced Akt-pT308 in different cancer cell lines (Supplementary Fig. 4a, b), leading to suppression of colony formation, anchorage-independent growth, and glucose uptake in cells (Supplementary Fig. 4c-f). Conversely, ectopic expression of SETDB1-WT, but not SETDB1-H1224K [27], induced Akt-pT308 in *Setdb1*-deficient MEFs (Fig. 3h). Collectively, these results indicate that the activation of Akt by SETDB1 appears to be methylation-dependent. Of note, consistent with a previous report [28], IHC staining revealed that compared to nevus tissues, SETDB1 is highly expressed in melanoma, especially in metastatic malignancies (Fig. 3i, j), and positively correlates with Akt1 K140-me3 and Akt-pS473 IHC signals (Fig. 3k-m).

SETDB1 is suggested to accelerate melanoma onset in cooperation with BRAF^{V600E} [28]. Given that coordinated actions of BRAF^{V600E} with activated Akt are required to promote melanomagenesis [33, 34], we employed a well-established human immortalized melanocyte (HIM) model [35] to examine whether SETDB1 modulates the activation of Akt. To this end, we found that concomitant activation of Akt and ERK signaling by SETDB1 and BRAF^{V600E} led to a marked enhancement of anchorage-independent cell growth, coupled with an increased Akt phosphorylation (Fig. 4a-c). Furthermore, deletion of endogenous *AKT1* in HEK293 cells compromised the oncogenic capability of SETDB1 to promote cell colony formation compared with parental cells (Fig. 4d-f).

More importantly, depletion of *SETDB1* resulted in decreased Akt phosphorylation and cellular malignant phenotypes in Akt1-WT expressing DLD1-*AKT1/2*$^{-/-}$ cells, but did not in Akt1-K140/142R expressing cells that already displayed relatively lower oncogenic capacity than WT cells (Fig. 4g-j). These results indicate that, Akt is likely one of the major downstream pathways through which SETDB1

exerts its oncogenic role. Consistently, re-introduction of constitutively active Akt1 (myr-Akt1) partially restored the colony formation capability of *SETDB1*-depleted A375 cells (Supplementary Fig. 4g, h). Furthermore, SETDB1 was capable of accelerating cell growth and colony formation in $AKT1^{WT}$, but not in the methylation-deficient $AKT1^{K140/142R}$ expressing HEK293 cells (Fig. 4k-n). Hence, our results suggest that SETDB1 may exert its oncogenic roles largely through Akt activation in a methylation-dependent manner.

SETDB1-mediated Akt methylation crosstalks with PI3K-mediated Akt phosphorylation

It is well established that Akt activation requires PI3K-dependent generation of PIP_3, which binds with the PH-motif of Akt at the plasma membrane [36-38]. We explored the temporal relationship between methylation-dependent and PI3K-dependent activation of Akt in cells. We showed that the interaction between Akt1 and SETDB1, and subsequent methylation of Akt, temporally coincided with Akt-pT308 following insulin or IGF stimulation (Fig. 5a and Supplementary Fig. 4i-k). Interestingly, PI3K inhibitors, but not Akt1 mutated variants (T308A and/or S473A), diminished the interaction of Akt1 with SETDB1, and decreased Akt1-K140 methylation in cells (Fig. 5b and Supplementary Fig. 4l, m), indicating that the PI3K pathway may function upstream of SETDB1-mediated Akt methylation to govern Akt activation. In further support of this notion, the non-PIP_3 binding mutation of Akt1-R25C [39], largely impaired the interaction between Akt and SETDB1, leading to a marked decrease in Akt1-K140 methylation (Fig. 5c, d).

To further pinpoint the potential role of PIP_3 in mediating the interaction between SETDB1 with Akt1-PH motif, we performed *in vitro* binding assays. Results suggest that the interaction of SETDB1 with Akt1-WT, but not Akt1-R25C, could be enhanced by PIP_3 *in vitro* (Fig. 5e and Supplementary Fig.

5a-e), indicating that PIP_3 binding could induce a conformation change of the PH motif, which facilitates Akt1 binding to SETDB1. Importantly, *PTEN* deficiency dramatically increased Akt1-K140 methylation coupled with increased Akt-pT308 (Supplementary Fig. 5f). Together, these data suggest that SETDB1 mediates Akt methylation likely in a PI3K catalytic activity-dependent manner.

Consistent with the finding that methylation of Akt1 could promote Akt-pT308, we observed that WT, but not the catalytically inactive SETDB1-H1224K, could induce the interaction of Akt1 with PDK1 in cells (Fig. 5f and Supplementary Fig. 5g). Moreover, the methylation-deficient Akt1 mutant (K140/142R) or depletion of *SETDB1* displayed an attenuated interaction with PDK1 compared with Akt1-WT (Fig. 5g, h and Supplementary Fig. 5h). In further support of the role for Akt methylation in controlling its activation, reducing Akt methylation by deleting *Setdb1* or introducing the Akt1-K140/142R mutation diminished the interaction of Akt1 with PIP_3 (Fig. 5i, j), and subsequently led to a marked decrease in association of Akt1 with the cell membrane (Fig. 5k, l and Supplementary Fig. 5i-m). These results indicate that methylated Akt1 may have a greater propensity to bind PIP_3 and translocate to the cellular membrane to achieve full activation. Thus, there is likely an intrinsic interplay between PI3K-mediated and SETDB1-mediated pathways to tightly control the spatial and temporal activation of Akt (Supplementary Fig. 5n).

Given that TRAF6-mediated Akt ubiquitination has a critical role in regulating Akt membrane translocation [15, 40, 41], we also found that WT, but not SETDB1-H1224K mutant, increased the interaction between Akt1 and TRAF6 (Supplementary Fig. 5o), resulting in an increase of Akt ubiquitination (Supplementary Fig. 5p). Furthermore, in comparison with Akt1-WT, Akt1-K140/142R displayed a reduced interaction with TRAF6 (Supplementary Fig. 5q), resulting in decreased Akt ubiquitination (Supplementary Fig. 5r). These findings suggest that SETDB1-induced methylation likely mediates the

interaction of Akt1 with TRAF6, and subsequent Akt ubiquitination may promote membrane translocation of methylated Akt (Supplementary Fig. 5n).

KDM4B demethylates and attenuates Akt kinase activity

Importantly, the Jumonji 2 (JMJD2, also termed KDM4) family proteins have been shown to function as specific erasers of SETDB1-mediated H3K9me3 [42], therefore, we assessed their ability to demethylate Akt1-K140me3. Notably, among all members of KDM4 family, KDM4A and KDM4B, and to a lesser extent KDM4C, were observed to interact with Akt1 (Fig. 6a and Supplementary Fig. 6a). Furthermore, *in vitro* demethylation assays [42] demonstrated that KDM4B-WT, but not the catalytically inactive KDM4B-H189A or other KDM4 members could efficiently erase the tri-methylation of Akt1 at K140 *in vitro* (Fig. 6b and Supplementary Fig. 6b-e) or in cells (Supplementary Fig. 6f). Moreover, the interaction between KDM4B and Akt1 was readily induced by enforcing expression of SETDB1-WT, but not its catalytic mutant (Fig. 6c). We further showed that Akt interacts with KDM4B through its Tudor domain (Supplementary Fig. 6g-j) in a methylation-dependent manner (Fig. 6d, e and Supplementary Fig. 6k, l). Hence, these data indicate that KDM4B might be a *bona fide* demethylase of Akt1.

KDM4B-depletion concomitantly increased Akt-pT308 and K140-me3 in multiple cell lines with Akt1-WT, but not those with $AKT1^{K140/142R}$ cells (Fig. 6f-h and Supplementary Fig. 6m-p). Strikingly, depletion of *KDM4A* displayed a reduction in Akt phosphorylation level (Supplementary Fig. 6q, r). In addition, depletion of *KDM4B*, but not *KDM4A*, largely enhanced Akt1 membrane translocation (Fig. 6i and Supplementary Fig. 6s). Furthermore, the interaction between Akt1 and KDM4B primarily occurred in the later phase of insulin stimulation (Supplementary Fig. 6t). This interaction was abolished by PI3K inhibitors (Fig. 6j). Consistent with the finding that KDM4B interacts with methylated-Akt, the

patient-derived mutation (Akt1-E17K) increased the interaction between Akt1 and KDM4B, while the non-PIP_3 binding mutation (Akt1-R25C) had the opposite effect (Fig. 6k). Clinically, KDM4B was expressed at relatively high levels in benign nevus compared with malignant melanoma (Supplementary Fig. 6u, v). This observation correlated with decreased Akt-pS473 and K140-me3 IHC signals (Supplementary Fig. 6u, w, x). Taken together, these data suggest that KDM4B might function as a negative regulator, antagonizing SETDB1-mediated methylation and activation of Akt.

SETDB1 is a potential therapeutic target of Akt-driven cancers

Given the critical role for the PI3K/Akt pathway in facilitating tumorigenesis, targeting hyperactive PI3K/Akt signaling by PI3K and/or Akt inhibitors have been pursued as promising anti-cancer therapies. However, relatively high-dose of these inhibitors induced cellular toxicity restricting their potential use as a treatment option in cancer patients [43, 44]. Thus, our finding that SETDB1 synergizes with PI3K to activate Akt kinase (Supplementary Fig. 7a) indicates that SETDB1 specific inhibitors could benefit cancer patients by targeting both H3K9-mediated epigenetic and PI3K/Akt oncogenic pathways. In support of this hypothesis, we showed that depletion of *SETDB1* significantly decrease A375 tumor growth *in vivo* (Fig. 7a-d), coupled with a reduction in H3K9me3 and Akt phosphorylation (Fig. 7e).

Consistent with a previous report [45], we found that Mithramycin A, an anti-neoplastic antibiotic, could markedly decrease SETDB1 expression and H3K9me3 in different cells. Mithramycin A subsequently led to reduced Akt methylation and phosphorylation (Fig. 7f and Supplementary Fig. 7b) and decreased the interaction of Akt1 and PDK1 (Fig. 7g). Biologically, Mithramycin A attenuated the phosphorylation and colony formation of cells expressing Akt1-WT, but not in *AKT1/2*-depleted DLD1 cells or *AKT1*$^{K140/142R}$-edited HEK293 cells (Fig. 7h-j and Supplementary Fig. 7c-e). To explore the

potential anti-tumor roles of Mithramycin A *in vivo*, we treated nude mice bearing A375 or DLD1 xenografted tumors, and showed that Mithramycin A could significantly decrease tumor growth compared with the vehicle treatment (Fig. 7k-n, and Supplementary Fig. 7f-i). Conceivably, the reduction of SETDB1 expression mediated by Mithramycin A decreased H3K9me3 and Akt phosphorylation (Supplementary Fig. 7j). Taken together, these results suggest that SETDB1 inhibition could possibly benefit cancer patients by repressing Akt oncogenic signaling in addition to its well characterized role in reprogramming the epigenome (Supplementary Fig. 7k).

DISCUSSION

In order to identify novel non-histone methylated proteins involved in oncogenic signaling pathways, we performed a mass spectrometry based high-throughput screen and detected numerous proteins modified by tri-methylation. We found that Akt1 could be methylated in its Linker region, which was enhanced during physiological conditions such as growth factor (Insulin or IGF) stimuli or pathological conditions such as Akt1-E17K mutation or *PTEN* deficiency. Absence of Akt methylation represses its kinase activity, and markedly decreases cell growth, glucose uptake, and tumorigenesis. More strikingly, methylation-deficient *Akt1* knock-in mice exhibit decreased body weight/size, which phenocopies *Akt1* knockout mice [46]. Importantly, we also observed that methylation-deficient *Akt1* knock-in could attenuate tumorigenesis in a carcinogen-induced skin tumor mouse model. Further investigations will be needed to determine whether knock-in methylation-deficient *Akt1* in genetic alteration models, such as $KRas^{G12D}$ mutation and *p53* depletion-mediated lung cancer models (*KP* mice) [47], could efficiently compromise the lung tumorigenesis through the decrease of Akt activity.

Among the known upstream signals, Akt1-T308 phosphorylation mediated by PDK1 is pivotal for Akt activation in a PIP_3 dependent fashion [48]. Here, we observed that SETDB1-mediated Akt methylation crosstalks with PI3K-mediated Akt phosphorylation. We suspect that PIP_3 binding to the PH domain of Akt could "unlock" the Akt auto-inhibition confirmation. The conformation change could be necessary for SETDB1 to interact with the Akt PH domain, and to methylate Akt in its exposed Linker region, in turn enhancing the interaction of Akt with its upstream kinase PDK1 (Supplementary Fig. 5n). We also observed that SETDB1 could enhance the interaction of Akt with TRAF6 to facilitate Akt ubiquitination, subsequent PIP_3 binding and membrane translocation (Supplementary Fig. 5n). Our model suggests a loop of Akt activation triggered by PIP_3 accumulation or Akt1-E17K mutation, which could be antagonized by

the demethylase KDM4B (Supplementary Fig. 5n). Additionally, the SETDB1-Tudor domain interacts with Akt1 mainly in the context of Akt1 Linker region being methylated. Thus, we speculate that methylation of the Linker region could be the primary modification that enhances SETDB1 interaction with Akt1, and sequentially promotes methylation of Akt on other lysine residues (such as K64 in Akt1 PH domain). Consistent with our findings, another group also revealed that SETDB1-mediated Akt1 tri-methylation at K64 plays important role for Akt interacting with and being ubiquitinated by TRAF6 to facilitate Akt membrane translocation and kinase activation (DOI from NCB). Therefore, the complicated connection and crosstalk of SETDB1-mediate different lysine methylation on Akt including K140/K142 in linker domain and K64 in PH domain warrants further investigation.

Finally, our study reveals that SETDB1 accelerates tumorigenesis largely through Akt activation in a methylation-dependent manner. As such, the function of SETDB1 is not restricted to the nucleus to repress gene transcription, but SETDB1 also methylates non-histone proteins in the cytoplasm such as Akt to activate its oncogenic functions.

Figure Legends

Fig. 1 Akt methylation promotes its activity and oncogenic functions

a, A schematic workflow of IAP-LC-mass spectrometry (MS)/MS experiments. OVCAR5 cell lysates were proteolytically digested to perform IAP-LC-MS/MS assays. **b**, Alignment of MS-characterized Akt1 putative methylation residues among different species, Akt2 and Akt3. **c-e**, Immunoblot (IB) analysis of whole cell lysates (WCL) derived from DLD1-*AKT1/2*$^{-/-}$ cells infected with indicated Akt1 encoding virus and selected with hygromycin (200 μg/ml) for 72 hrs before harvesting (**c**). Resulting cells were subjected to colony formation and soft agar assays (**d**). The experiment was repeated twice, independently, with similar results (**c**,**d**). Relative colony numbers were quantified (**e**). Error bars are mean ± s.e.m, n = 3 independent repeats. **f-h**, Cells generated in (**c**) were subjected to mice xenograft assays. Tumor sizes were monitored (**g**), and dissected tumors were weighed (**h**). Error bars are mean ± s.e.m, n = 7 independent repeats. **i-k**, K140R and K142R mutations of Akt1 were genetically engineered in HEK293 cells by the CRISPR/CAS9-based technique. Resulting cells were serum-starved for 12 hrs, and harvested for IB analysis at different time points after stimulation with insulin (100 nM) (**i**). Cells generated in (**i**) were assessed for proliferation (**j**) and colony formation (**k**) assays. Error bars are mean ± s.e.m, n = 3 independent repeats. The experiment was repeated twice, independently, with similar results (**i**,**k**). **l**,**m**, Cells generated in (**i**) were subjected to the measurement of glucose uptake and lactate production with Fluorescence-activated cell sorting (FACS) and lactate strips, respectively. The detection of glucose uptake and relative numbers of lactate production were plotted (**l**,**m**). Error bars are mean ± s.e.m, n = 3 independent repeats. Two-tailed unpaired Student's *t* test was performed in **e**,**h**,**k**,**m** to calculate the *P* value. Two-way ANOVA analysis was performed in **f**,**j**,**l** to calculate the *P* value. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

Fig. 2 Methylation deficient *Akt1* knock-in mice display reduced body size/weight and resist to chemical carcinogen-induced skin tumorigenesis *in vivo*

a, Mice derived from the same litter were imaged at age of 4 weeks old. **b**, The mice derived from the age of 4 weeks were weighed (including 15 male mice with 5 WT, 5 heterogeneous, and 5

homogeneous-*Akt1*$^{K140/142R}$ genetic background status). Error bars are mean ± s.e.m, n = 5 independent repeats. *P* values were calculated using two-tailed unpaired Student's *t* test. **c**, The mice in (**b**) were euthanized and their organs were dissected and weighed. Error bars are mean ± s.e.m, n = 5 independent repeats. *P* values were calculated using two-tailed unpaired Student's *t* test. **d**, IB analysis of WCL derived from livers or hearts of WT (+/+), *Akt1*$^{K140/142R}$-knock-in heterogeneous (+/KI) or homogeneous (KI/KI) mice from the same litter at age of 4 weeks. The experiment was repeated twice, independently, with similar results. **e**, Graphic representation of H&E and IHC staining of heart and liver tissues derived from WT or *Akt1*$^{K140/142R}$-KI mice. Scale bar, 50 μm. This experiment was repeated twice, independently, with similar results. **f-i**, The side view of 12-weeks old mice derived from WT or *Akt1*$^{K140/142R}$ knock-in mice were treated with chemical carcinogen (DMBA following with TPA) (n=16 for WT mice; n=11 for Akt1-K140/142-KI mice). (**f**). The neoplasm lesions were arrowed. The tumor incidence (**g**) and lesion numbers (**h**) were calculated and plotted. Error bars are mean ± s.e.m. After treatment 12 weeks with DMBA/TPA, the mice were euthanized and the H&E and IHC staining were performed (**i**). Scale bar, 1mm. The experiment was repeated twice, independently, with similar results. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

Fig. 3 SETDB1 methylates Akt on K140 and K142 to promote its kinase activity

a,**b**,**d**, IB analysis of immunoprecipitates (IP) and WCL derived from A375 and OVCAR5 (**a**) or HEK293 cells transfected with indicated constructs (**b**) or *AKT1*$^{K140/142R}$-KI cells stably expressed WT or catalytically inactive SETDB1-H1224K (**d**). IgG was used as a negative control. **c**, *In vitro* methylation assays were performed with recombinant His-Akt1 proteins purified from insect cells as substrates and purified Flag-SETDB1 from HEK293T cells as the source of methyltransferase in the presence/absence of ^{3}H-SAM. **e**, IB analysis of IP products and WCL derived from A375 cells lentivirally transfected with shRNAs against *SETDB1*. Resulting cells were selected with puromycin for 72 hrs before harvesting. **f**, *Setdb1* conditional knockout MEFs were treated with or without 4-OHT (500 nM) for 48 hrs to deplete endogenous *Setdb1*, then resulting cells were serum-starved for another 20 hrs and stimulated with insulin (100 nM) for 15 min before being harvested and subjected to IP and IB analysis. **g**, IB analysis of IP products and WCL derived from HEK293 cells transfected with indicated constructs. **h**, *Setdb1* conditional knockout MEFs were infected with WT or H1224K-SETDB1 encoding virus and selected

with puromycin for 72 hrs, then treated with or without 4-OHT (500 nM) for another 48 hrs before harvesting for IB analysis. **i-m**, Image illustration of the immunohistochemistry (IHC) staining for SETDB1, pS473-Akt1 and K140-me3-Akt1 in melanoma TMA (**i**,**k**). Scale bar, 50 μM. The distribution of SETDB1 staining was plotted (**j**) (n=97). The correlations of pS473-Akt or K140-me3-Akt with SETDB1 were plotted (**l**,**m**) (n=95). All Western-blots above in (**a**,**b**,**d**,**e**,**f**,**g**,**h**) were repeated twice, independently, with similar results. All *P* values were calculated using *Chi-Square* test in **j**,**l**,**m**. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

Fig. 4 Oncogenic function of SETDB1 depends on the activation of Akt

a-**c**, Human immortalized melanocytes (HIM) were lentivirally infected with indicated constructs, and selected with puromycin and hygromycin for 72 hrs before harvesting for IB analysis (**a**). Resulting cells were subjected to soft agar assays (**b**). The experiment were repeated twice, independently, with similar results (**a**,**b**). Relative colony numbers were quantified (**c**). **d**-**f**, IB analysis of CRISPR/CAS9-mediated *AKT1* knockout and parental HEK293 cells which were lentivirally infected with the constructs encoding SETDB1 (**d**). Resulting cells were subjected to colony formation assays (**e**). The experiment were repeated twice, independently, with similar results (**d**,**e**). Relative colony numbers were quantified (**f**). **g**-**j**, DLD1-$AKT1/2^{-/-}$ cells were infected with virus encoding WT or mutated Akt1, and selected with hygromycin for 72 hrs. Resulting cells were lentivirally infected with shRNA against *SETDB1* (with shCtr as a negative control) and selected with puromycin for 72 hrs, and were harvested for IB analysis (**g**), cell proliferation (**h**), colony formation and soft agar (**i**) assays. The experiment was repeated twice, independently, with similar results (**g**,**i**). Relative colony numbers were quantified (**j**). **k**-**n**, $AKT1^{K140/142R}$-edited and parental HEK293 cells were lentivirally infected with SETDB1-WT or SETDB1-H1224K encoding constructs and selected with puromycin for 72 hrs before harvesting for IB analysis (**k**). Resulting cells were subjected to proliferation (**l**) and colony formation (**m**) assays. The experiment was repeated twice, independently, with similar results (**k**,**m**). Relative colony numbers were quantified (**n**). All Error bars are mean ± s.e.m, n = 3 independent repeats. Two-tailed unpaired Student's *t* test was performed in **c**,**f**,**j**,**n** to calculate *P* values. Two-way ANOVA analysis was performed in **h**,**l** to

calculate the *P* value. Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

Fig. 5 SETDB1-medidated methylation of Akt synergizes with PI3K to activate Akt

a,**b**, A375 cells were serum-starved for 20 hrs, then stimulated with insulin (50 nM) at different time points (**a**) or post-treatment of various PI3K inhibitors (**b**) before harvesting for IP and IB analysis. **c**,**d**, IB analysis of IP products and WCL derived from HEK293 cells transfected with indicated constructs stimulated without (**c**) or with IGF (100 ng/ml) (**d**) before harvesting. **e**, *In vitro* binding assays were performed with recombinant GST-Akt1 protein purified from mammalian cells, and flag beads bound SETDB1. The binding was performed in $4^{o}C$ for 4 hrs incubated with or without PIP_3 (20 μM) and subjected to IB analysis. **f**-**h**, IB analysis of Akt1-IP and WCL derived from HEK293 cells infected with indicated SETDB1 encoding constructs (**f**), $AKT1^{K140/142R}$ and its parental HEK293 cells (**g**) and *SETDB1* depleted A375 cells (**h**). **i**,**j**, IB analysis of PIP_3 pull-down products and WCL derived from *Setdb1* conditional knockout MEFs treated with or without 4-OHT (500 nM) for 48 hrs (**i**) or from HEK293 cells transfected with indicated constructs (**j**). Where indicated, empty beads (Ctr) serve as a negative control. **k**,**l**, IB analysis of cell fractionations separated from *Setdb1* conditional knockout MEFs treated with or without 4-OHT (500 nM) for 48 hrs (**k**) or from $AKT1^{K140/142R}$-edited and parental HEK293 cells (**l**). All Western-blots above were repeated twice, independently, with similar results. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

Fig. 6 KMD4B demethylates Akt to inhibit Akt kinase activity

a,**c**-**e**, IB analysis of Akt1-IP products and WCL derived from A375, OVACR5 cells (**a**), SETDB1 expressing HEK293 cells (**c**), *AKT1*$^{K140/142R}$-edited and parental HEK293 cells (**d**) and *SETDB1*-depleted A375 cells (**e**). IgG was used as a negative control. **b**, IB analysis of *in vitro* de-methylation assays performed with synthetic Akt1-K140-me3 peptides as substrate, and bacterially purified catalytic domain of KDM4B as the source of demethylase. **f**, IB analysis of WCL derived from primary *Kdm4b* conditional knockout MEFs infected with or without phage-Cre for 48 hrs before harvesting. **g**, A375 cells were lentivirally infected with shRNA against *KDM4B*. Resulting cells were serum starved for 20 hrs, then stimulated with IGF (100 ng/ml) before harvesting for IP and IB analysis. **h**, IB analysis of IP products and WCL derived from A375 cells infected with lentivirus against *KDM4B*. **i**, IB analysis of cell fractionations separated from A375 cells lentivirally infected with shRNA against *KDM4B* or *KDM4A*. **j**,**k**, HEK293 cells were transfected with indicated constructs and treated with different PI3K inhibitors for 1 hr (**j**) before subjected to GST pull-down assay and IB analysis. All Western-blots above were repeated twice, independently, with similar results. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

Fig. 7 Deficiency of SETDB1 inhibits Akt kinase activity and oncogenic function

a-**d**, *SETDB1*-depleted A375 and control cells were subjected to mice xenograft assays. Tumor sizes were monitored (**a**,**b**). Tumors were dissected (**c**) and tumor mass were weighed (**d**). Error bars are mean ± s.e.m, n = 7 independent repeats. *P* values were calculated by using two-way ANOVA analysis (**b**) and two-tailed unpaired Student's *t* test (**d**). **e**, The phosphorylation status of Akt1 (pT308-Akt) and methylation of H3K9 (H3K9me3) were detected by IB analysis with WCL derived from recovered xenografted tumors. The experiment was repeated twice, independently, with similar results. **f**, IB analysis

of IP products and WCL derived from A375 cells treated with different doses of Mithramycin A for 72 hrs before harvesting. The experiment was repeated twice, independently, with similar results. **g**, HEK293 cells were transfected with indicated constructs and treated with different doses of Mithramycin A for 72 hrs before harvesting for GST pull-down assays and IB analysis. The experiment was repeated twice, independently, with similar results. **h**-**j**, *AKT1*$^{K140/142R}$-edited and parental HEK293 cells were treated with different doses of Mithramycin A for 72 hrs and subjected to IB analysis (**h**). Meantime, resulting cells were subjected to colony formation (**i**) assay. The experiment was repeated twice, independently, with similar results (**h**,**i**). Relative colony numbers were quantified (**j**). Error bars are mean ± s.e.m, n = 3 independent repeats. Two-tailed unpaired Student's *t* test was performed in (**j**) to calculate *P* values. **k**-**n**, Mithramycin A treatment reduced *in vivo* tumorigenesis of xenografted A375 cells. When the tumors of xenografted A375 cells reached 100 mm^3, the mice were treated with Mithramycin A (0.2 mg/kg) or PBS (as a negative control). Tumor sizes were monitored (**k**,**l**) and tumor mass were weighed and presented in (**m**,**n**). Error bars are mean ± s.e.m, n = 8 independent repeats. *P* values were calculated by using two-way ANOVA analysis (**l**) and two-tailed unpaired Student's *t* test (**n**). Detailed statistical tests are described in the Methods. Scanned images of unprocessed blots are shown in Supplementary Fig. 8.

REFERENCES

1. Jones, P.A., Issa, J.P. & Baylin, S. Targeting the cancer epigenome for therapy. *Nat Rev Genet* **17**, 630-641 (2016).
2. You, J.S. & Jones, P.A. Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell* **22**, 9-20 (2012).
3. Glaser, K.B. HDAC inhibitors: clinical update and mechanism-based potential. *Biochem Pharmacol* **74**, 659-671 (2007).
4. Fahy, J., Jeltsch, A. & Arimondo, P.B. DNA methyltransferase inhibitors in cancer: a chemical and therapeutic patent overview and selected clinical studies. *Expert Opin Ther Pat* **22**, 1427-1442 (2012).
5. Delmore, J.E. *et al.* BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* **146**, 904-917 (2011).
6. Kelly, T.K., De Carvalho, D.D. & Jones, P.A. Epigenetic modifications as therapeutic targets. *Nat Biotechnol* **28**, 1069-1078 (2010).
7. Daigle, S.R. *et al.* Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood* **122**, 1017-1025 (2013).
8. Biggar, K.K. & Li, S.S. Non-histone protein methylation as a regulator of cellular signalling and function. *Nat Rev Mol Cell Biol* **16**, 5-17 (2015).
9. Chuikov, S. *et al.* Regulation of p53 activity through lysine methylation. *Nature* **432**, 353-360 (2004).
10. Guo, A. *et al.* Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. *Mol Cell Proteomics* **13**, 372-387 (2014).
11. Saddic, L.A. *et al.* Methylation of the retinoblastoma tumor suppressor by SMYD2. *J Biol Chem* **285**, 37733-37740 (2010).
12. Vanhaesebroeck, B. & Alessi, D.R. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* **346 Pt 3**, 561-576 (2000).
13. Manning, B.D. & Toker, A. AKT/PKB Signaling: Navigating the Network. *Cell* **169**, 381-405 (2017).
14. Ozes, O.N. *et al.* NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature* **401**, 82-85 (1999).
15. Yang, W.L. *et al.* The E3 ligase TRAF6 regulates Akt ubiquitination and activation. *Science* **325**, 1134-1138 (2009).
16. Liu, P. *et al.* Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. *Nature* **508**, 541-545 (2014).
17. Guo, J. *et al.* pVHL suppresses kinase activity of Akt in a proline-hydroxylation-dependent manner. *Science* **353**, 929-932 (2016).
18. Mazur, P.K. *et al.* SMYD3 links lysine methylation of MAP3K2 to Ras-driven cancer. *Nature* **510**, 283-287 (2014).
19. Yoshioka, Y. *et al.* SMYD3-mediated lysine methylation in the PH domain is critical for activation of AKT1. *Oncotarget* **7**, 75023-75037 (2016).
20. Cong, L. *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819-823 (2013).
21. Cho, H., Thorvaldsen, J.L., Chu, Q., Feng, F. & Birnbaum, M.J. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J Biol Chem* **276**,

38349-38352 (2001).

22. Nasti, T.H. *et al.* A murine model for the development of melanocytic nevi and their progression to melanoma. *Mol Carcinog* **55**, 646-658 (2016).
23. Gao, H. *et al.* Akt/PKB interacts with the histone H3 methyltransferase SETDB1 and coordinates to silence gene expression. *Mol Cell Biochem* **305**, 35-44 (2007).
24. Liu, T. *et al.* Histone methyltransferase SETDB1 maintains survival of mouse spermatogonial stem/progenitor cells via PTEN/AKT/FOXO1 pathway. *Biochim Biophys Acta* **1860**, 1094-1102 (2017).
25. Cha, T.L. *et al.* Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. *Science* **310**, 306-310 (2005).
26. Cerami, E. *et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* **2**, 401-404 (2012).
27. Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G. & Rauscher, F.J., 3rd SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev* **16**, 919-932 (2002).
28. Ceol, C.J. *et al.* The histone methyltransferase SETDB1 is recurrently amplified in melanoma and accelerates its onset. *Nature* **471**, 513-517 (2011).
29. Macgregor, S. *et al.* Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3. *Nat Genet* **43**, 1114-1118 (2011).
30. Fei, Q. *et al.* Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. *Nat Commun* **6**, 8651 (2015).
31. Wong, C.M. *et al.* Up-regulation of histone methyltransferase SETDB1 by multiple mechanisms in hepatocellular carcinoma promotes cancer metastasis. *Hepatology* **63**, 474-487 (2016).
32. Carpten, J.D. *et al.* A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* **448**, 439-444 (2007).
33. Cao, J. *et al.* MC1R is a potent regulator of PTEN after UV exposure in melanocytes. *Mol Cell* **51**, 409-422 (2013).
34. Dankort, D. *et al.* Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat Genet* **41**, 544-552 (2009).
35. Garraway, L.A. *et al.* Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* **436**, 117-122 (2005).
36. Cantley, L.C. The phosphoinositide 3-kinase pathway. *Science* **296**, 1655-1657 (2002).
37. Alessi, D.R. *et al.* Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol* **7**, 261-269 (1997).
38. Stephens, L. *et al.* Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science* **279**, 710-714 (1998).
39. Franke, T.F., Kaplan, D.R., Cantley, L.C. & Toker, A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science* **275**, 665-668 (1997).
40. Chan, C.H. *et al.* The Skp2-SCF E3 ligase regulates Akt ubiquitination, glycolysis, herceptin sensitivity, and tumorigenesis. *Cell* **149**, 1098-1111 (2012).
41. Cederquist, C.T. *et al.* Systemic insulin sensitivity is regulated by GPS2 inhibition of AKT ubiquitination and activation in adipose tissue. *Mol Metab* **6**, 125-137 (2017).
42. Whetstine, J.R. *et al.* Reversal of histone lysine trimethylation by the JMJD2 family of histone

demethylases. *Cell* **125**, 467-481 (2006).
43. Luo, J., Manning, B.D. & Cantley, L.C. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. *Cancer Cell* **4**, 257-262 (2003).
44. Hennessy, B.T., Smith, D.L., Ram, P.T., Lu, Y. & Mills, G.B. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov* **4**, 988-1004 (2005).
45. Ryu, H. *et al.* ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington's disease. *Proc Natl Acad Sci U S A* **103**, 19176-19181 (2006).
46. Chen, W.S. *et al.* Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. *Genes Dev* **15**, 2203-2208 (2001).
47. DuPage, M., Dooley, A.L. & Jacks, T. Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase. *Nat Protoc* **4**, 1064-1072 (2009).
48. Downward, J. Mechanisms and consequences of activation of protein kinase B/Akt. *Curr Opin Cell Biol* **10**, 262-267 (1998).

SUPPLEMENTARY INFORMATION is available in the online version of the paper.

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