Pathology - Research and Practice 215 (2019) 1054–1060

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Knockdown of KDM1B inhibits cell proliferation and induces apoptosis of pancreatic cancer cells

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ARTICLE INFO

Keywords:
Pancreatic cancer
KDM1B
Proliferation
Cell apoptosis
Signaling pathways

ABSTRACT

Pancreatic cancer (PC) is one of the common malignant tumors in digestive tract with a high fatality rate. The oncogenic role of lysine-specific demethylase1 (LSD1/KDM1 A) has been well recognized in PC. While, the role of its homolog LSD2 (KDM1B) in regulating PC progression is poorly understood. In this study, we attempted to evaluate the functional role of KDM1B in PC cells. The expression of KDM1B was detected by immunohistochemistry and immunoblotting in PC tissues and cells. Lentivirus-mediated shRNA was applied to silence KDM1B in PANC-1 and SW1990 cells. Cell proliferation was measured by MTT and Celigo assay. Cell apoptosis was determined by both Caspase-Glo®3/7 assay and Flow cytometry. Intracellular signaling molecules were detected using a PathScan intracellular signaling array kit. In this study, we found KDM1B was highly expressed in PC tissues compared to paracancerous tissues. Moreover, elevated expression of KDM1B was detected in PC cell lines (BxPC-3, CFPAC-1, PANC-1 and SW1990) as compared with a normal human pancreatic duct epithelial cell line (HPDE6-C7). Further investigations revealed that KDM1B knockdown significantly inhibited PC cell proliferation. Furthermore, the apoptosis of PANC-1 and SW1990 cells was significantly increased after KDM1B knockdown. Notably, the activations of p-ERK1/2, p-Smad2, p-p53, cleaved PARP, cleaved Caspase-3, cleaved Caspase-7, p-eIF2a and Survivin were promoted by KDM1B knockdown, while IkBa was suppressed. Taken together, our findings provided new insights into the critical and multifaceted roles of KDM1B in the regulation of cell proliferation and apoptosis, and offered a potentially novel target in preventing the progression of PC.

1. Introduction

Pancreatic cancer (PC) is one of the common malignant tumors in digestive tract with a high fatality rate, which is difficult to diagnose and treat [1]. The operative mortality is high and the prognosis is extremely poor because of the high recurrence rate [2,3]. Therefore, an increasing understanding of the complex molecular basis of PC and exploring novel therapeutic targets may contribute to the clinical treatment of this aggressive malignancy.

In the recent years, more and more evidence support that dysregulated epigenetic regulatory processes play a central role in cancer onset and progression [4–6]. Reversible histone methylation is an important process within epigenetic regulation, and the investigations of its role in cancer have led to the identification of lysine methyltransferases and demethylases as promising targets for cancer therapy [7–9]. Histone demethylases (KDMs) are a family of enzymes that catalyze the removal of methyl groups not only from histones but other proteins as well, playing a pivotal role in dynamic regulation of numerous chromatin functions such as gene transcription, chromatin stability, DNA replication and repair [10–12]. The first histone demethylase to be discovered was lysine-specific demethylase 1 (LSD1), which are homologues of the flavin-containing amine oxidases [13,14]. Further researches identified an additional family of histone demethylases, which can be divided into five subfamilies (KDM2/7, KDM3, KDM4, KDM5, and KDM6 subfamilies), belonging to the Fe^{2+} and α-ketoglutarate-dependent Jumonji C-terminal domain family (JMJD) [15–17]. As a deeper understanding of their involvement in transcriptional regulation is gained, KDMs are becoming increasingly interesting targets for drug development.

KDM1 family includes KDM1 A (LSD1) and KDM1B (LSD2), which

Abbreviations: PC, pancreatic cancer; KDMs, demethylases; LSD1, lysine-specific demethylase 1; JMJD, Jumonji domain-containing proteins; IHC, immunohistochemistry; MTT, 3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide; FCM, flow cytometry; PARP, poly-(ADP-ribose) polymerase

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https://doi.org/10.1016/j.prp.2019.02.014
Received 31 December 2018; Received in revised form 23 January 2019; Accepted 26 February 2019

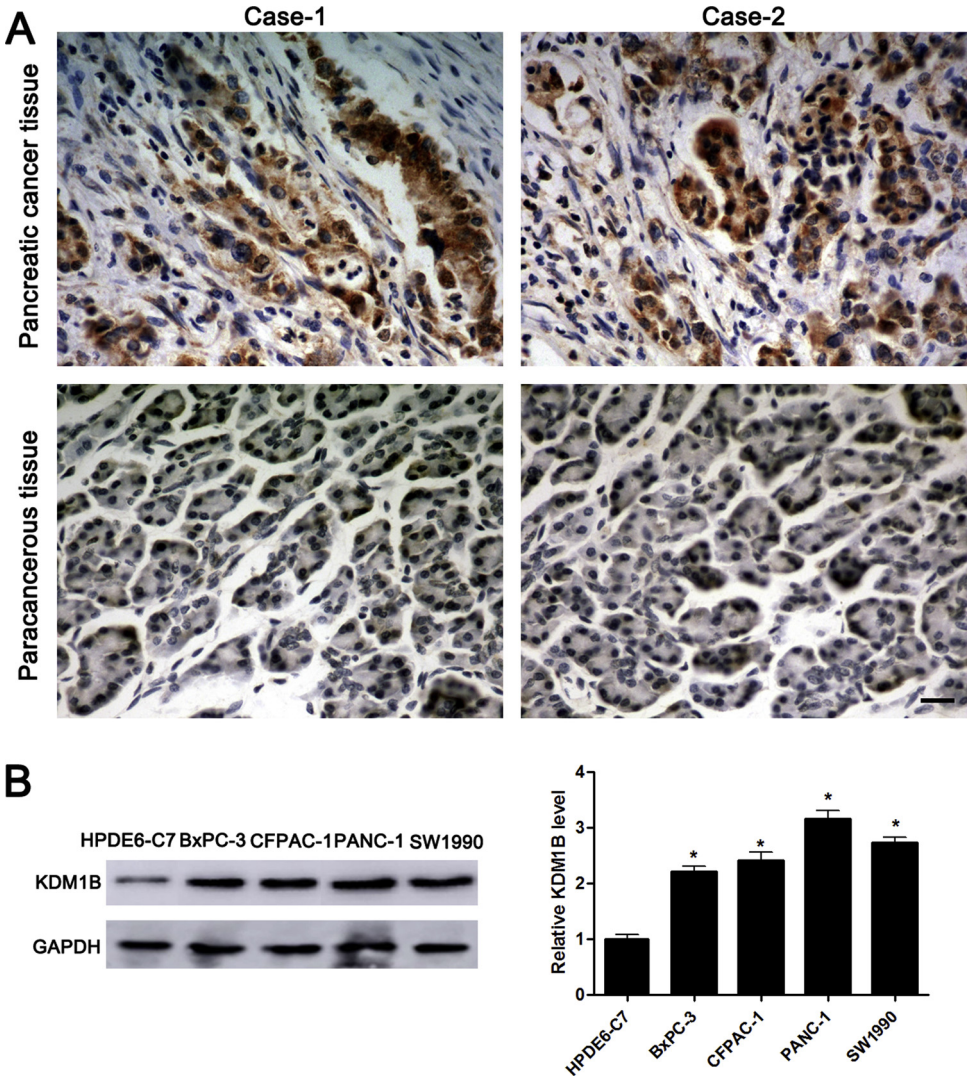


Fig. 1. The expression of KDM1B in PC. (A) The representative immunohistochemical staining of KDM1B in PC and paracancerous tissues. Scale bar: 20 μm. (B) The expressions of KDM1B among HPDE6-C7, BxPC-3, CFPAC-1, PANC-1 and SW1990 cell lines. n = three independent repeats, *P < 0.05 by ANOVA.

scores an overall 33% identity in the SWIRM domain, the FAD coenzyme-binding motif and the C-terminal amine oxidase domains [13,18]. In contrast to LSD1, LSD2 lacks the "tower domain", which is instrumental to the tight LSD1-CoREST association [18–20]. However, LSD2 possesses a zinc finger domain in the N-terminal region, which may confer biochemical and biological properties distinct from those of LSD1. KDM1 A is involved in a wide array of biological processes including cell proliferation, chromosome segregation and embryonic development. Recently, KDM1 A has been identified as a potential target for cancer therapeutics. Although KDM1B shares similar substrate specificity with KDM1 A, it is evident that instead of functioning as a transcriptional repressor, KDM1B is important for transcriptional elongation factors and phosphorylated RNA polymerase II [21,22]. KDM1B tends to associates predominantly with the gene bodies of actively transcribed genes, but does not assemble the promoter as KDM1 A [20]. These findings suggest that KDM1 A and KDM1B likely interact with different protein domain and show quite distinct genomic distribution profiles. According to the discoveries from the high-throughput sequencing projects of primary human tumor samples, mRNA expression levels of both KDM1 A and KDM1B were greatly increased in several different types of tumors, including multiple myeloma, esophageal squamous cell carcinomas, renal cell carcinomas, breast and colorectal cancer and glioblastoma [23,24]. KDM1B has been linked to numerous important biological function including genomic imprinting, transcription regulation, regulation of somatic cell reprogramming, DNA methyltransferases and growth factor signaling [25–28]. However, it is still not clear whether KDM1B is also involved in the development of PC.

The purpose of this study is to investigate KDM1B expression and its functional significance in PC. We used lentivirus-mediated shRNA to down-regulate KDM1B expression in PC cell lines *in vitro* and detected the changes of cell proliferation and apoptosis. These studies provided a novel insight into the functional role of KDM1B in PC progression.

2. Materials and methods

2.1. Tissue specimens

Twenty pairs of PC tissues and matched paracancerous tissues were obtained from 1st Affiliated Hospital of Xi'an Jiaotong University. The use of human tissues was approved by This study was reviewed and approved by the Ethic Committee of 1 st Affiliated Hospital of Xi'an Jiaotong University in accordance with the guidelines outlined in the Declaration of Helsinki. All patients signed an informed consent.

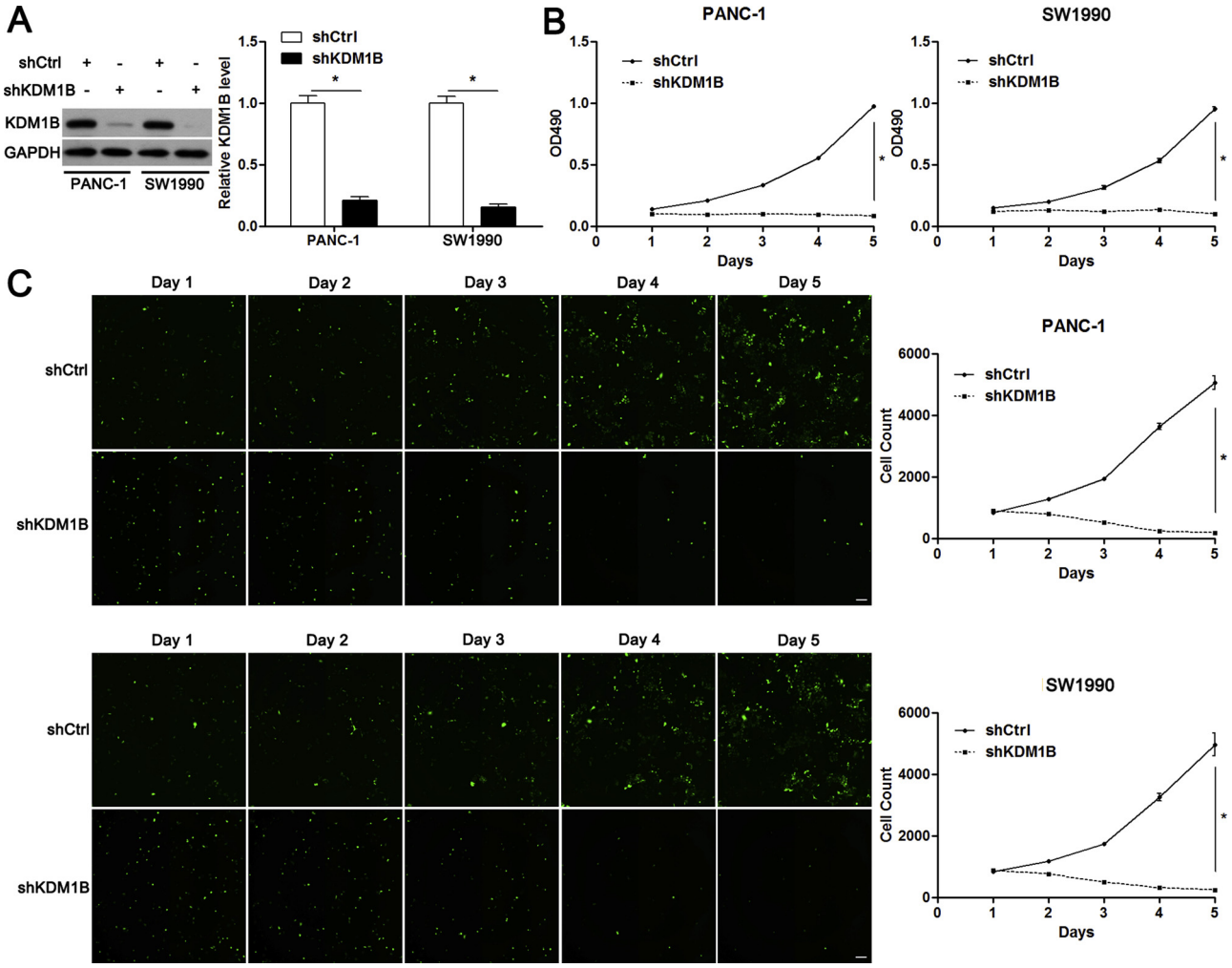


Fig. 2. KDM1B knockdown suppresses PC cell proliferation. (A) PANC-1 and SW1990 cells that were infected with lenti-viruses mediated shCtrl or shKDM1B were detected by immunoblotting for KDM1B expression. n = three independent repeats, *P < 0.05 by *t*-test (B) MTT assays indicated that KDM1B knockdown significantly restrained PC cell viability. n = three independent repeats, *P < 0.05 by ANOVA. (C) The number of PANC-1 and SW1990 cells were obviously reduced after KDM1B knockdown. Scale bar: 100 μm, n = three independent repeats, *P < 0.05 by ANOVA.

2.2. Cell lines

Human pancreatic cancer cell lines (BxPC-3, CFPAC-1, PANC-1, SW1990) and a normal human pancreatic duct epithelial cell line HPDE6-C7 were purchased from the Shanghai Cell Bank of the Chinese Academy of Science (Shanghai, China). Cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Corning, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; Ausbian, Australia) at 37 °C in a 5% CO_2 incubator.

2.3. Immunohistochemistry (IHC)

IHC was performed using the SP kit (SP-9000, ZSGB-BIO, Beijing, China) according to the manufacturer's protocols. After dewaxing and hydration, sections were heated in citrate buffer (pH 6.0, Sigma Aldrich, USA) in a microwave oven for 16 min for antigen retrieval. The sections were incubated in 3% hydrogen peroxide for 10 min at room temperature, and blocked with 5% goat serum (Bioss Biotechnology) for 20 min at 37 °C. Rabbit anti-KDM1B polyclonal antibody (HPA031269; Sigma Aldrich) was added and sections were incubated overnight at 4 °C. Horseradish peroxidase-conjugated secondary antibody (Bioss Biotechnology) were added. The proteins were visualized by diaminobenzidine. Staining intensity was graded: 0 (absence of staining), 1 (weakly stained), 2 (moderately stained), and 3 (strongly stained). The percentage of positive tumor cells was scored: 0 (0–5%), 1 (6–25%), 2 (26–50%) and 3 (50–100%). The sum scores ≥3 points were considered as positive [29].

2.4. Recombinant lentiviral vector production and cell infection

To create the shRNA target site, the complementary DNA sequence (ACTGGCTTTACTACAGAAA) of KDM1B was designed by Shanghai GeneChem Co., Ltd. (Shanghai, China) using the full-length human sequence (GenBank no. NM_153042). The KDM1B hairpin oligonucleotides were synthesized and inserted into the pGV112-GFP (GeneChem Co. Ltd.) lentiviral vector. Lentivirus particles were prepared as previously described [30]. For lentiviral infection, PANC-1 and SW1990 cells were cultured in 6-well plates. The KDM1B-siRNA lentivirus (shKDM1B) or negative control lentivirus (shCtrl) was added according to a multiplicity of infection (MOI 1:15). At 3 days post-infection, the cells were observed for presence of the GFP marker with a fluorescence microscope (MicroPublisher 3.3RTV; Olympus, Tokyo, Japan). Then, the cells were harvested and the knockdown efficiency was analyzed by western blot analysis.

2.5. Western blot analysis

While on ice, cell lysates were incubated for 10–15 min in ice-cold

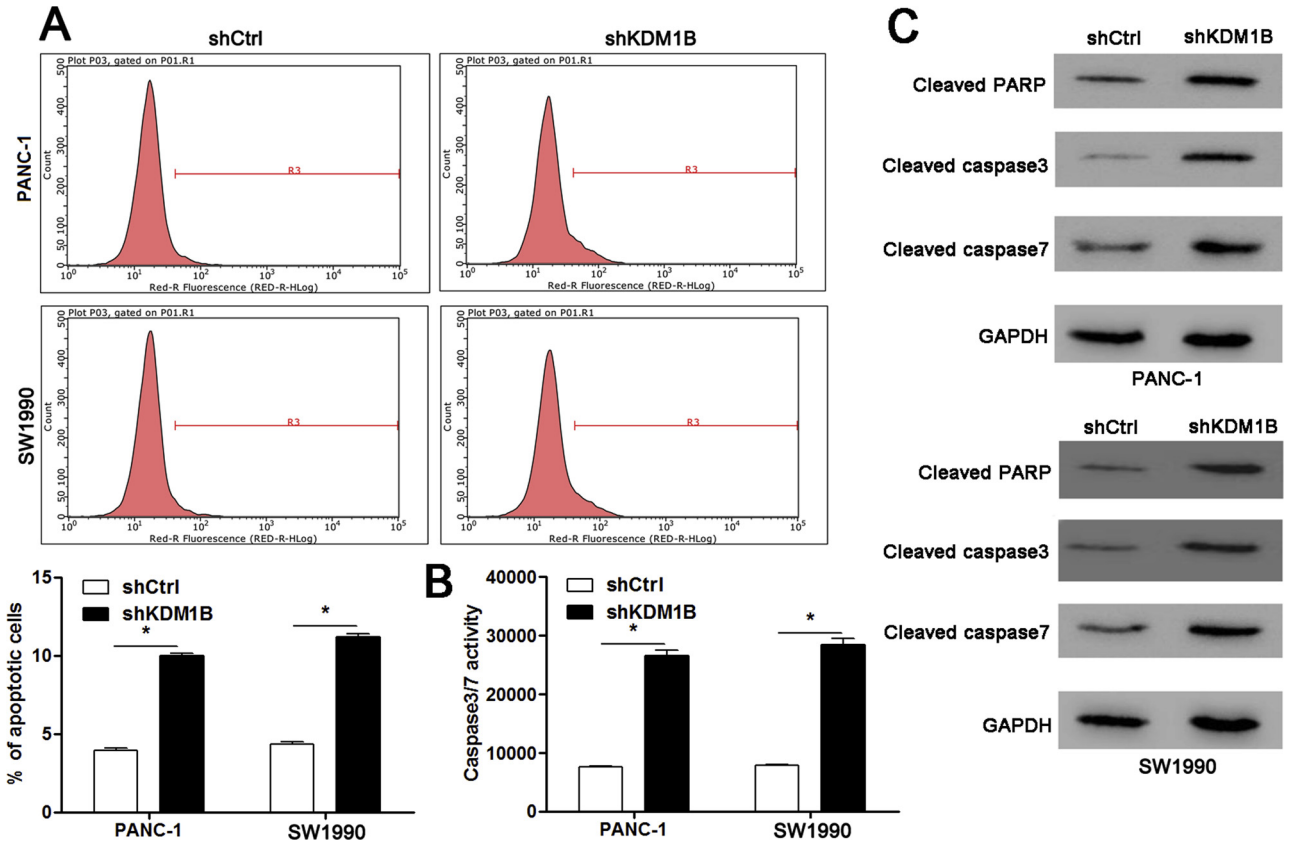


Fig. 3. KDM1B knockdown induces the apoptosis of PC cells. (A) Flow cytometry analysis indicated that KDM1B knockdown significantly induced PC cell apoptosis. n = three independent repeats, *P < 0.05 by *t*-test (B) The activity of Caspase3/7 was obviously increased after KDM1B knockdown in PANC-1 and SW1990 cells. n = three independent repeats, *P < 0.05 by *t*-test (C) KDM1B knockdown increased the levels of cleaved PARP, cleaved caspase3 and cleaved caspase7 in PANC-1 and SW1990 cells.

lysis buffer (100 mM Tris, pH 6.8, 2% β-mercaptoethanol, 20% glycerol, 4% SDS). The lysates were centrifuged at 12,000 x g for 15 min at 4 °C, and the supernatants were collected. Protein concentration was determined using a BCA protein assay kit (Beyotime, Beijing, China). An equal amount of total protein from each sample was partially separated in a 10% SDS-PAGE gel and blotted onto PVDF membranes. Membranes were incubated with KDM1B (ab193080; Abcam, Cambridge, MA, USA), cleaved PARP (ab32064; Abcam), Caspase-3 (ab32351; Abcam), Caspase-7 (ab32522; Abcam) or GAPDH primary antibodies (sc-32233; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight, followed by horseradish peroxidase (HRP)-conjugated bovine anti-mouse/rabbit IgG (sc-2371 and sc-2370; Santa Cruz Biotechnology) secondary antibody at room temperature. Enhanced chemiluminescence (ECL) reagent (ECL-Plus/kit; Amersham, Piscataway, NJ, USA) was used for detection. The amount of GAPDH detected was used as the protein loading internal control.

2.6. Cell proliferation assay

After being infected with the shCtrl lentivirus or shKDM1B lentivirus, PANC-1 and SW1990 cells were seeded in 96-well plates at a concentration of 2500 cells/well and incubated for 5 days at 37 °C with 5% CO_2. The cells were counted each day using the Celigo® Image Cytometer (Nexcelom, USA).At 1, 2, 3, 4, or 5 days post-infection, the cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml; Promega, Shanghai, China) at a final concentration of 0.5 mg/ml for 4 h. After discarding the supernatants, 150 µl of dimethyl sulfoxide (DMSO; Sigma-Aldrich Co., LLC, St. Louis, MO, USA) was added to each well. The plates were read at 490 nm using an ELISA reader (Tecan Infinite, Männedorf, Switzerland). All experiments were performed in triplicate.

2.7. Analysis of apoptosis

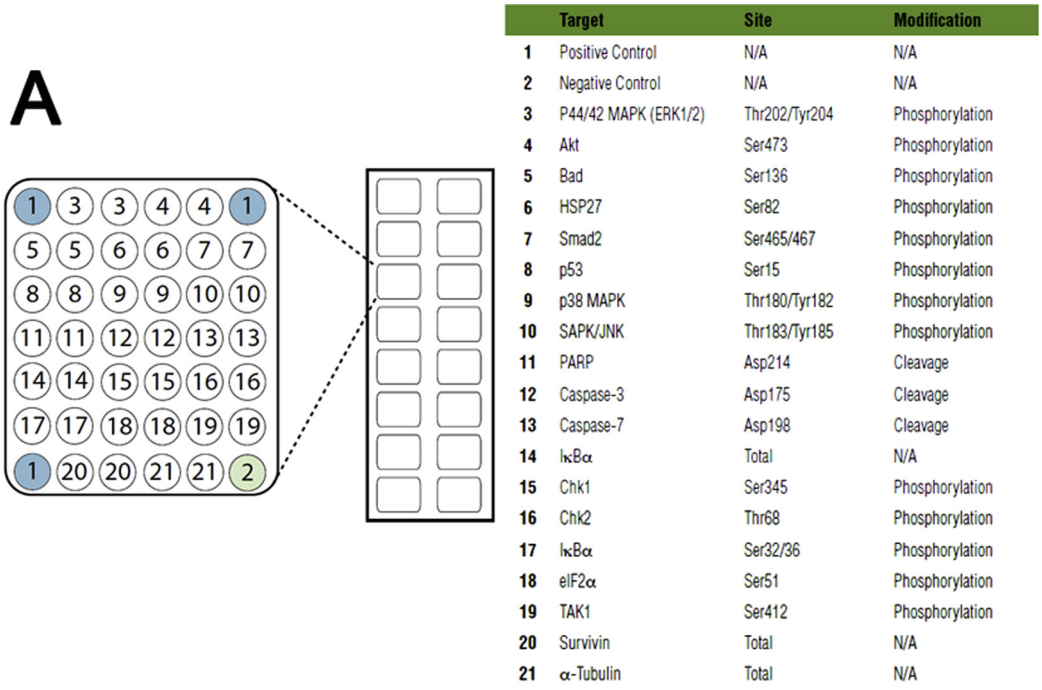
Flow cytometry (FCM) was used to measure apoptosis and was performed as previously described [31]. After lentivirus infection for 3 days, PANC-1 and SW1990 cell were seeded in a volume of 2 ml at a density of 5×10^5 cells/well in 6-cm dishes, respectively. The cells were collected and washed twice with ice-cold D-Hanks and 1X binding buffer. Cell suspension (200 µl) and Annexin V-APC (10 µl, eBioscience, San Diego, CA, USA) were thoroughly mixed and incubated in darkness at room temperature for 10–15 min. All rates of apoptosis were measured by FCM within 1 h. Each experiment was performed in triplicate.

2.8. Assessment of caspase-3 and caspase-7 activities

The caspase-3 and caspase-7 activities of PANC-1 and SW1990 cells were evaluated using the Caspase-Glo® 3/7 assay (Promega, Madison, WI, USA), according to the manufacturer's instructions. The PANC-1 and SW1990 cells (1.5×10^4 cells/well) were seeded in 96-well plates. At 72 h post-transfection, the Caspase-Glo® 3/7 reagent was added to each well, the plates were shaken gently at 300–500 rpm for 30 s and incubated for 2 h at room temperature. Then the luminescence was measured using plate-reading luminometer. The data analyzed were of triplicate samples.

2.9. Intracellular signaling array

Cell lysates were prepared as mentioned above. Intracellular signaling molecules were detected using a PathScan® Antibody Array Kit



	Target	Site	Modification
1	Positive Control	N/A	N/A
2	Negative Control	N/A	N/A
3	P44/42 MAPK (ERK1/2)	Thr202/Tyr204	Phosphorylation
4	Akt	Ser473	Phosphorylation
5	Bad	Ser136	Phosphorylation
6	HSP27	Ser82	Phosphorylation
7	Smad2	Ser465/467	Phosphorylation
8	p53	Ser15	Phosphorylation
9	p38 MAPK	Thr180/Tyr182	Phosphorylation
10	SAPK/JNK	Thr183/Tyr185	Phosphorylation
11	PARP	Asp214	Cleavage
12	Caspase-3	Asp175	Cleavage
13	Caspase-7	Asp198	Cleavage
14	IκBα	Total	N/A
15	Chk1	Ser345	Phosphorylation
16	Chk2	Thr68	Phosphorylation
17	IκBα	Ser32/36	Phosphorylation
18	eIF2α	Ser51	Phosphorylation
19	TAK1	Ser412	Phosphorylation
20	Survivin	Total	N/A
21	α-Tubulin	Total	N/A

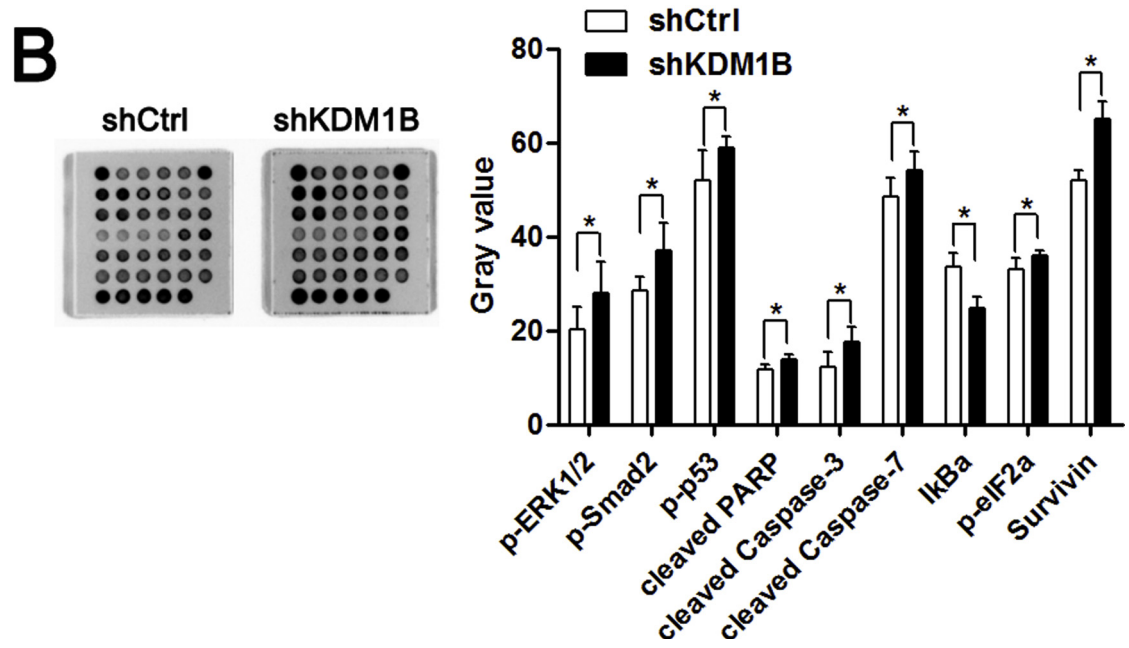


Fig. 4. KDM1B knockdown modulates multiple signaling molecules in PANC-1 cells. (A) The distribution and detailed information for Intracellular signaling array. (B) The activations of p-ERK1/2, p-Smad2, p-p53, cleaved PARP, cleaved Caspase-3, cleaved Caspase-7, p-eIF2a and Survivin were promoted by KDM1B knockdown, while IkBa was suppressed. n = three independent repeats, *P < 0.05 by *t*-test.

(Cell Signaling Technology) according to the manufacturer's procedure.

2.10. Statistical analyses

Data for each group are presented as the mean ± SD from at least three independent experiments. Statistical analyses were performed using GraphPad Prism version 5. Value of *p < 0.05 was deemed statistically significant.

3. Results

3.1. KDM1B was highly expressed in PC

The expression KDM1B between PC tissues and paracancerous tissues was detected by IHC. KDM1B was located at both cytoplasm and nuclear. Our results indicated that 13 of 20 PC tissues showed KDM1B positive expression whereas KDM1B positive signal was detected in only 6 of 20 paracancerous tissues (65.0% *versus* 30.0%, P = 0.027, Fig. 1A). Next, KDM1B expressions were measured in HPDE6-C7, BxPC-3, CFPAC-1, PANC-1 and SW1990 cell lines by immunoblotting. As a

result, the levels of KDM1B in PC cell lines were markedly higher than that in HPED6-C7 cells ($P < 0.05$, Fig. 1B). Next, analysis of TCGA data from starBase V3.0 [32] revealed that there was no significant difference of KDM1B mRNA expression between PC and normal pancreatic tissues (Supplementary Fig. 1A). While, the expression of KDM1B mRNA in PC tissues was markedly higher than that in normal pancreatic tissues based on GEO dataset (GSE62165; $P < 0.0001$, Supplementary Fig. 1B). Furthermore, TCGA data indicated that PC patients with high KDM1B level had an obvious but not significant shorter overall survival compared to KDM1B low-expressing patients (Supplementary Fig. 1C).

3.2. KDM1B knockdown suppresses PC cell proliferation

We chose PANC-1 and SW1990 cells as the typical PC cell lines for further experiments, in which KDM1B is relatively high abundant. To examine the effect of KDM1B on cell growth, KDM1B knockdown was performed in PANC-1 and SW1990 cells ($P < 0.05$. Fig. 2A and Supplementary Fig. 2). shCtrl- and shKDM1B-infected PANC-1 and SW1990 cells were reseeded in 96-well plates and analyzed at 1, 2, 3, 4, and 5 days post-infection. As illustrated in Fig. 2B, although shCtrl and shKDM1B cells had similar *in vitro* growth on days 1, 2, and 3, the shKDM1B cells had significantly reduced *in vitro* growth on days 4 and 5 ($P < 0.05$). The effect of KDM1B protein reduction on PC cell proliferation was also determined by Celigo assay. shCtrl cells exhibited extensive proliferation at 5 days post-infection, while the number of shKDM1B cells slightly decreased ($P < 0.05$, Fig. 2C). These results revealed that KDM1B knockdown significantly inhibited the proliferation of PANC-1 and SW1990 cells.

3.3. KDM1B knockdown increases PC cell apoptosis

To test whether KDM1B expression affects PC cell death, we knocked down KDM1B and measured apoptosis. Annexin V staining followed by FCM was used to determine cell apoptosis. As shown in Fig. 3A, apoptosis was significantly increased in the shKDM1B group compared to the shCtrl group ($P < 0.05$). The effects of KDM1B expression on apoptosis in PANC-1 and SW1990 cells was also assessed by evaluating caspase-3 and caspase-7 activities. As shown in Fig. 3B, PANC-1 and SW1990 cells with shKDM1B infected became significantly high activity of caspase3/7 compared to that of shCtrl ($P < 0.05$). Moreover, KDM1B knockdown increased the levels of cleaved PARP, cleaved caspase3 and cleaved caspase7 in PC cells (Fig. 3C). These results indicated that KDM1B expression is an important determinant of apoptosis in PANC-1 and SW1990 cells.

3.4. KDM1B knockdown affects multiple signaling molecules

To further illuminate the molecular mechanisms by which shKDM1B affects PC cell growth, a PathScan® Antibody Array Kit was used to detect the changes of signaling molecules in PANC-1 cells after shKDM1B knockdown. As shown in Fig. 4, the phosphorylation of ERK1/2, Smad2, p53, cleaved PARP, cleaved Caspase-3, cleaved Caspase-7, eIF2a and Survivin were up-regulated in shKDM1B-treated cells, while IkBa was down-regulated in shKDM1B-treated cells, respectively ($P < 0.05$). The data indicated that shKDM1B knockdown could significantly inhibit growth of PC cells *via* activations of ERK1/2, Smad2, p53, cleaved PARP, cleaved Caspase-3, cleaved Caspase-7, eIF2a and Survivin, and blockade of IkBa.

4. Disscusion

The Multidisciplinary Pancreatic Cancer Clinic developed a pancreatic cancer treatment plan based on the unique needs of the patient, only approximately 20% of the tumors were found to be operable or resectable. Therefore, it is necessary for us to look for the specific indicator and diagnosis of pancreatic cancer in the early stage. Previous studies have proven that epigenetic modifications play an important role in pancreatic cancer [33,34]. In the present study, we evaluated the functional significance of KDM1B in PC and its potential as a therapeutic target or a biomarker. We demonstrated that overexpression of KDM1B was a frequent event in PC. Next, we used lentivirus-mediated shRNA to silence KDM1B expression in PC cells. Knockdown of KDM1B significantly reduced cell proliferation in PANC-1 and SW1990 cells as suggested by both MTT and Celigo tests. Moreover, KDM1B knockdown increased the activity of Caspase3/7, indicating that KDM1B may decrease the apoptosis trend of PC cells. Flow cytometry also identified the inhibition effect of KDM1B on apoptosis of PANC-1 and SW1990 cells. These results suggested that KDM1B might play a critical role in promoting PC cell proliferation. Our data were in accordance with a previous report showing the knockdown of KDM1B in breast cancer cells, which led to a significant reduction in cell proliferation [35].

Furthermore, to illuminate the molecular mechanisms by which KDM1B affects PC cell growth, we detected modifications of some molecules associated with cell apoptosis, growth and survival control in PANC-1 cells after KDM1B knockdown. It may be logical to consider that higher levels of apoptosis in a tumor could lead to a better prognosis. Apoptosis mediated by either the intrinsic or extrinsic pathways results in cleavage of Caspase-3, Caspase-7. Caspase-3 is rarely mutated in pancreatic cancer and is therefore an ideal marker for measuring apoptosis in these tumors [36]. Our studies have validated the use of active caspase-3 staining as a marker for apoptosis. To further validate apoptosis within the tumor, cleavage of the poly (ADP-ribose) polymerase (PARP) catalyzed by caspase-3 was also measured. The activations of all these three apoptosis-related molecules were increased by KDM1B knockdown, which strongly confirmed that KDM1B plays a key role in PC apoptosis *in vitro*. To the best of our knowledge, this is the first *in vitro* study concerning the effects of KDM1B against PC cell growth.

Previous studies reviewed the anti-apoptotic effect of NF-κB [37]. In the present study, when shKDM1B was used to interfere with the expression of KDM1B, the IkBa was greatly down-regulated. These results suggested that the inhibition of NF-κB signaling served a critical role in the inhibitory effect of KDM1B against apoptosis in PANC-1 cells. Previous studies demonstrated that survivin was a small molecular, acting as a genuine apoptosis inhibitor [38], which contradicted our results in the antibody chip. In our studies, the expression of survivin was up-regulated, accompanying the activation of p-ERK/eIF2a signaling pathway, whereas the apoptosis was increased. On the contrary, high level of p-ERK/eIF2a contributes to the occurrence and development of hepatocyte cell apoptosis in alcoholic liver injury rats [39]. Therefore, further verification of p-ERK/eIF2a signaling pathway should be taken.

The antibody chip brought up another hint that KDM1B knockdown induced up-regulation of p-p53 and p-Smad2. The p53 pathway is targeted for inactivation in most human cancers either directly or indirectly, highlighting its critical function as a tumor suppressor gene. Upon activation, p53 mediates a growth-suppressive effect on cells by blocking the cell cycle or it can lead cells to undergo programmed cell death primarily by binding to particular DNA sequences and activating transcription of specific genes. Furthermore, previous study has demonstrated that TGF-β/Smad signaling is operational in PC [40]. We for the first time found that KDM1B suppressed apoptosis in PC cells probably *via* p53/Smad signaling.

In summary, we elucidated the *in vitro* activities of KDM1B in regulation of PC cell proliferation and apoptosis. These studies provided a novel insight into the previously unrecognized roles of KDM1B in human PC cells. We showed for the first time that KDM1B knockdown attenuated proliferation and induced apoptosis of PC cells. In the future, better understanding of epigenetic downstream target genes and pathways controlled by KDM1B would aid in developing novel small molecule inhibitors which might confer selective effects against PC.

5. Conclusions

To conclude, we investigated the expression of KDM1B between PC and matched paracancerous tissues, and elucidated the functional role of KDM1B and its underlying molecular mechanisms in PC cells. Our study revealed that overexpression of KDM1B was a frequent event in PC tissues. Functionally, KDM1B knockdown exerted a tumor suppressive role by inhibition of PC cell proliferation and inducing apoptosis. Alterations of p-ERK1/2, p-Smad2, p-p53, cleaved PARP, cleaved Caspase-3, cleaved Caspase-7, p-eIF2a, Survivin and IkBa were detected after KDM1B knockdown in PANC-1 cells. Our results may provide a novel theoretical and experimental basis for the pathogenesis of PC, and identify novel treatment targets.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81402186) and Shaanxi provincial social development project (2016SF-183).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.02.014.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer Statistics, 2017, CA Cancer J. Clin. 67 (1) (2017) 7–30.
[2] J.E. Ying, L.M. Zhu, B.X. Liu, Developments in metastatic pancreatic cancer: is gemcitabine still the standard? World J. Gastroenterol. 18 (8) (2012) 736–745.
[3] C.W. Michalski, M. Erkan, N. Huser, M.W. Muller, M. Hartel, H. Friess, J. Kleeff, Resection of primary pancreatic cancer and liver metastasis: a systematic review, Dig. Surg. 25 (6) (2008) 473–480.
[4] Z. Liu, Y. Wang, C. Dou, M. Xu, L. Sun, L. Wang, B. Yao, Q. Li, W. Yang, K. Tu, Q. Liu, Hypoxia-induced up-regulation of VASP promotes invasiveness and metastasis of hepatocellular carcinoma, Theranostics 8 (17) (2018) 4649–4663.
[5] Q. Xu, J. Tu, C. Dou, J. Zhang, L. Yang, X. Liu, K. Lei, Z. Liu, Y. Wang, L. Li, H. Bao, J. Wang, K. Tu, HSP90 promotes cell glycolysis, proliferation and inhibits apoptosis by regulating PKM2 abundance via Thr-328 phosphorylation in hepatocellular carcinoma, Mol. Cancer 16 (1) (2017) 178.
[6] K. Tu, W. Yang, C. Li, X. Zheng, Z. Lu, C. Guo, Y. Yao, Q. Liu, Fbxw7 is an independent prognostic marker and induces apoptosis and growth arrest by regulating YAP abundance in hepatocellular carcinoma, Mol. Cancer 13 (2014) 110.
[7] L. Morera, M. Lubbert, M. Jung, Targeting histone methyltransferases and demethylases in clinical trials for cancer therapy, Clin. Epigenetics 8 (2016) 57.
[8] R. Popovic, J.D. Licht, Emerging epigenetic targets and therapies in cancer medicine, Cancer Discov. 2 (5) (2012) 405–413.
[9] Z. Liu, Y. Wang, C. Dou, L. Sun, Q. Li, L. Wang, Q. Xu, W. Yang, Q. Liu, K. Tu, MicroRNA-1468 promotes tumor progression by activating PPAR-gamma-mediated AKT signaling in human hepatocellular carcinoma, J. Exp. Clin. Cancer Res. 37 (1) (2018) 49.
[10] J. Dabin, A. Fortuny, S.E. Polo, Epigenome maintenance in response to DNA damage, Mol. Cell 62 (5) (2016) 712–727.
[11] K.K. Li, C. Luo, D. Wang, H. Jiang, Y.G. Zheng, Chemical and biochemical approaches in the study of histone methylation and demethylation, Med. Res. Rev. (2010) n/a-n/a.
[12] S.M. Kooistra, K. Helin, Molecular mechanisms and potential functions of histone demethylases, nature reviews, Mol. Cell Biol. 13 (5) (2012) 297–311.
[13] Y. Shi, F. Lan, C. Matson, P. Mulligan, J.R. Whetstine, P.A. Cole, R.A. Casero, Histone demethylation mediated by the nuclear amine oxidase homolog LSD1, Cell 119 (7) (2004) 941–953.
[14] Y. Chen, Y. Yang, F. Wang, K. Wan, K. Yamane, Y. Zhang, M. Lei, Crystal structure of human histone lysine-specific demethylase 1 (LSD1), Proc. Natl. Acad. Sci. U. S. A. 103 (38) (2006) 13956–13961.
[15] Y. Tsukada, J. Fang, H. Erdjument-Bromage, M.E. Warren, C.H. Borchers, P. Tempst, Y. Zhang, Histone demethylation by a family of JmjC domain-containing proteins, Nature 439 (7078) (2006) 811–816.
[16] R.J. Klose, K. Yamane, Y. Bae, D. Zhang, H. Erdjument-Bromage, P. Tempst, J. Wong, Y. Zhang, The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36, Nature 442 (7100) (2006) 312–316.
[17] P.A. Cloos, J. Christensen, K. Agger, A. Maiolica, J. Rappsilber, T. Antal, K.H. Hansen, K. Helin, The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3, Nature 442 (7100) (2006) 307–311.
[18] A. Karytinos, F. Forneris, A. Profumo, G. Ciossani, E. Battaglioli, C. Binda, A. Mattevi, A novel mammalian flavin-dependent histone demethylase, J. Biol. Chem. 284 (26) (2009) 17775–17782.
[19] F. Chen, H. Yang, Z. Dong, J. Fang, P. Wang, T. Zhu, W. Gong, R. Fang, Y.G. Shi, Z. Li, Y. Xu, Structural insight into substrate recognition by histone demethylase LSD2/KDM1b, Cell Res. 23 (2) (2013) 306–309.
[20] R. Fang, A.J. Barbera, Y. Xu, M. Rutenberg, T. Leonor, Q. Bi, F. Lan, P. Mei, G.-C. Yuan, C. Lian, J. Peng, D. Cheng, G. Sui, U.B. Kaiser, Y. Shi, Y.G. Shi, Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation, Mol. Cell 39 (2) (2010) 222–233.
[21] L.J. Core, J.T. Lis, Transcription regulation through promoter-proximal pausing of RNA polymerase II, Science 319 (5871) (2008) 1791–1792.
[22] Q. Zhang, S. Qi, M. Xu, L. Yu, Y. Tao, Z. Deng, W. Wu, J. Li, Z. Chen, J. Wong, Structure-function analysis reveals a novel mechanism for regulation of histone demethylase LSD2/AOF1/KDM1b, Cell Res. 23 (2) (2013) 225–241.
[23] G.L. Dalgliesh, K. Furge, C. Greenman, L. Chen, G. Bignell, A. Butler, H. Davies, S. Edkins, C. Hardy, C. Latimer, J. Teague, J. Andrews, S. Barthorpe, D. Beare, G. Buck, P.J. Campbell, S. Forbes, M. Jia, D. Jones, H. Knott, C.Y. Kok, K.W. Lau, C. Leroy, M.L. Lin, D.J. McBride, M. Maddison, S. Maguire, K. McLay, A. Menzies, T. Mironenko, L. Mulderrig, L. Mudie, S. O'Meara, E. Pleasance, A. Rajasingham, R. Shepherd, R. Smith, L. Stebbings, P. Stephens, G. Tang, P.S. Tarpey, K. Turrell, K.J. Dykema, S.K. Khoo, D. Petillo, B. Wondergem, J. Anema, R.J. Kahnoski, B.T. Teh, M.R. Stratton, P.A. Futreal, Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes, Nature 463 (7279) (2010) 360–363.
[24] G. van Haaften, G.L. Dalgliesh, H. Davies, L. Chen, G. Bignell, C. Greenman, S. Edkins, C. Hardy, S. O'Meara, J. Teague, A. Butler, J. Hinton, C. Latimer, J. Andrews, S. Barthorpe, D. Beare, G. Buck, P.J. Campbell, J. Cole, S. Forbes, M. Jia, D. Jones, C.Y. Kok, C. Leroy, M.L. Lin, D.J. McBride, M. Maddison, S. Maquire, K. McLay, A. Menzies, T. Mironenko, L. Mulderrig, L. Mudie, E. Pleasance, R. Shepherd, R. Smith, L. Stebbings, P. Stephens, G. Tang, P.S. Tarpey, R. Turner, K. Turrell, J. Varian, S. West, S. Widaa, P. Wray, V.P. Collins, K. Ichimura, S. Law, J. Wong, S.T. Yuen, S.Y. Leung, G. Tonon, R.A. DePinho, Y.T. Tai, K.C. Anderson, R.J. Kahnoski, A. Massie, S.K. Khoo, B.T. Teh, M.R. Stratton, P.A. Futreal, Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer, Nat. Genet. 41 (5) (2009) 521–523.
[25] D.N. Ciccone, H. Su, S. Hevi, F. Gay, H. Lei, J. Bajko, G. Xu, E. Li, T. Chen, KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints, Nature 461 (7262) (2009) 415–418.
[26] D. van Essen, Y. Zhu, S. Saccani, A feed-forward circuit controlling inducible NF-kappaB target gene activation by promoter histone demethylation, Mol. Cell 39 (5) (2010) 750–760.
[27] S.L. Lin, D.C. Chang, C.H. Lin, S.Y. Ying, D. Leu, D.T. Wu, Regulation of somatic cell reprogramming through inducible mir-302 expression, Nucleic Acids Res. 39 (3) (2011) 1054–1065.
[28] T.A. Katz, Y. Huang, N.E. Davidson, R.C. Jankowitz, Epigenetic reprogramming in breast cancer: from new targets to new therapies, Ann. Med. 46 (6) (2014) 397–408.
[29] Q. Li, C. Wang, Y. Wang, L. Sun, Z. Liu, L. Wang, T. Song, Y. Yao, Q. Liu, K. Tu, HSCs-derived COMP drives hepatocellular carcinoma progression by activating MEK/ERK and PI3K/AKT signaling pathways, J. Exp. Clin. Cancer Res. 37 (1) (2018) 231.
[30] C. Lois, E.J. Hong, S. Pease, E.J. Brown, D. Baltimore, Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors, Science 295 (5556) (2002) 868–872.
[31] D. Wlodkowic, J. Skommer, Z. Darzynkiewicz, Flow cytometry-based apoptosis detection, Methods Mol. Biol. 559 (2009) 19–32.
[32] J.H. Li, S. Liu, H. Zhou, L.H. Qu, J.H. Yang, starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data, Nucleic Acids Res. 42 (Database issue) (2014) D92–7.
[33] S. Yokoyama, M. Higashi, S. Kitamoto, M. Oeldorf, U. Knippschild, M. Kornmann, K. Maemura, H. Kurahara, E. Wiest, T. Hamada, I. Kitazono, Y. Goto, T. Tasaki, T. Hiraki, K. Hatanaka, Y. Mataki, H. Taguchi, S. Hashimoto, S. Batra, A. Tanimoto, S. Yonezawa, M. Hollingsworth, Aberrant methylation of MUC1 and MUC4 promoters are potential prognostic biomarkers for pancreatic ductal adenocarcinomas, Oncotarget 7 (27) (2016) 42553–42565.
[34] S. Zagorac, S. Alcala, G. Fernandez Bayon, T. Bou Kheir, M. Schoenhals, A. Gonz??lez-Neira, M. Fernandez Fraga, A. Aicher, C. Heeschen, B. Sainz, DNMT1 inhibition reprograms pancreatic cancer stem cells via upregulation of the miR-17-92 cluster, Cancer Res. 76 (15) (2016) 4546–4558.
[35] L. Chen, S. Vasilatos, Y. Qin, T. Katz, C. Cao, H. Wu, N. Tasdemir, K. Levine, S. Oesterreich, N. Davidson, Y. Huang, Functional characterization of lysine-specific demethylase 2 (LSD2/KDM1B) in breast cancer progression, Oncotarget 8 (47) (2017) 81737–81753.
[36] P. Noble, M. Vyas, A. Al-Attar, S. Durrant, J. Scholefield, L. Durrant, High levels of cleaved caspase-3 in colorectal tumour stroma predict good survival, Br. J. Cancer 108 (10) (2013) 2097–2105.
[37] N. Perkins, Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway, Oncogene 25 (51) (2006) 6717–6730.
[38] D.S. O'Connor, D. Grossman, J. Plescia, F. Li, H. Zhang, A. Villa, S. Tognin, P.C. Marchisio, D.C. Altieri, Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin, Proc. Natl. Acad. Sci. U. S. A. 97 (24) (2000) 13103–13107.
[39] X. Han, J. Wang, L. Wang, P. Zheng, G. Ji, [Role of PERK/eIF2a signaling pathway in hepatocyte apoptosis of alcoholic liver injury rats], Zhonghua Gan Zang Bing Za Zhi 18 (10) (2010) 768–772.
[40] Y. Togashi, H. Sakamoto, H. Hayashi, M. Terashima, M. de Velasco, Y. Fujita, Y. Kodera, K. Sakai, S. Tomida, M. Kitano, A. Ito, M. Kudo, K. Nishio, Homozygous deletion of the Activin A receptor, type IB gene is associated with an aggressive cancer phenotype in pancreatic cancer, Mol. Cancer 13 (2014) 126.