Int J Clin Exp Pathol 2015;8(9):11140-11148
www.ijcep.com /ISSN:1936-2625/IJCEP0011182

Original Article

Decitabine induces G2/M cell cycle arrest by suppressing p38/NF-κB signaling in human renal clear cell carcinoma

Donghao Shang[1], Tiandong Han[1], Xiuhong Xu[1], Yuting Liu[2]

[1]*Department of Urology, Friendship Hospital, Capital Medical University, Beijing 100050, China;* [2]*Department of Pathology, Capital Medical University, Beijing 100069, China*

Received June 8, 2015; Accepted July 21, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: Objective: The anti-neoplastic effects of decitabine, an inhibitor of DNA promoter methylation, are beneficial for the treatment of renal cell carcinoma (RCC); however, the mechanism of action of decitabine is unclear. We analyzed gene expression profiling and identified specific pathways altered by decitabine in RCC cells. Methods: Four human RCC cell lines (ACHN, Caki-1, Caki-1, and A498) were used in this study; growth suppression of RCC cells by decitabine was analyzed using the WST-1 assay. Apoptosis and cell cycle arrest were examined using flow cytometric analysis. Gene expression of RCC cells induced by decitabine was evaluated with cDNA microarray, and potential biological pathways were selected using Ingenuity Pathway Analysis. The activity of the p38-NF-κB pathway regulated by decitabine was confirmed by Western blotting. Results: Decitabine suppresses the proliferation of RCC cells *in vitro*. Although decitabine did not significantly induce apoptosis, decitabine caused cell cycle arrest at G2/M in a dose-dependent manner. Gene expression regulated by decitabine in RCC cells was investigated using microarray analysis. Ubiquitin carboxyl terminal hydrolase 1 (UCHL1), interferon inducible protein 27 (IFI27), and cell division cycle-associated 2 (CDCA2) may be involved in growth suppression of RCC cells by decitabine. The phosphorylation of p38-NF-κB pathway was suppressed by decitabine in RCC cells. Conclusions: We investigated gene expression profiling and pathways modulated by decitabine in RCC cells. Decitabine was shown to suppress the growth of RCC cells via G2/M cell cycle arrest and the p38-NF-κB signaling pathway may play a role in the anti-neoplastic effect of decitabine in RCC cells.

Keywords: Renal cell carcinoma, decitabine, apoptosis, cell cycle arrest, gene expression, p38-NF-κB pathway

Introduction

Renal cell carcinoma (RCC) is the most common tumor involving the adult kidney. RCC is characterized by diverse histologic features and clinical phenotypes, including clear cell RCC (ccRCC), chromophobe RCC, and papillary RCC (PRCC), which originate from the epithelium of renal tubules [1, 2]. ccRCC is the major histologic sub-type, accounting for > 75% of all cases [3]. Due to technologic advances, an increased number of patients with RCC are diagnosed at an early stage and < 10% will succumb due to this malignancy [4]. Approximately 30% of patients diagnosed with localized RCC will undergo relapse after nephrectomy. Moreover, patients with metastatic RCC have a poor 5-year survival rate, ranging from 0-20% [5].

Hypermethylation of cancer-associated genes has been observed in various malignancies, and DNA methylation plays a critical role in human carcinogenesis [6]. Frequently, gene methylation, such as death-associated protein kinase (DAPK) 1, von Hippel-Lindau (VHL) tumor suppressor, tumor suppressor protein RDA32 (RASSFIA), and tissue inhibitor of metalloproteinase (TIMP) 3, have been confirmed in human RCC [7, 8]. The anti-neoplastic effects of 5-aza-2'-deoxycytidine (decitabine), an inhibitor of DNA promoter methylation, appear to be beneficial for treatment of different tumors [9, 10]. Moreover, the effects of concomitant inhibition with decitabine and chemotherapeutic drugs were also examined in human tumors. Lou et al. [11] suggested that the gefitinib and decitabine combination exerts synergistic anti-cancer

activity and might be considered as a novel therapeutic regimen for treating colon cancer. Another study indicated that the combination of decitabine and panitumumab is well-tolerated and has activity against metastatic colorectal cancer [12]. We also investigated the growth suppression of decitabine and chemotherapeutic drugs in urinary tract carcinoma, and showed that combination decitabine and paclitaxel (PTX) caused synergistic growth suppression of RCC and prostate carcinoma (PC), suggesting that decitabine and PTX might be a novel strategy to improve the clinical response rate of RCC and PC [13, 14]. In addition, we found that decitabine could significantly increase the susceptibility of transitional cell carcinoma to cisplatin, and synergistic growth suppression by two agents was confirmed in all TCC cell lines tested [15].

The mechanism of action of decitabine is complicated and controversial. Even though decitabine, a DNA methylation inhibitor, has been reported to suppress tumor cell proliferation by inducing genome-wide demethylation or reactivating the expression of specific methylated genes [16, 17], another study indicated that decitabine enhances anti-neoplastic activity by causing DNA damage [18]. A recent study showed that decitabine treatment can significantly increase CD80 gene expression, and the CTL response elicited by the overexpression of CD80 in a variety of human cancer cells suggests that decitabine is also involved in cancer immunotherapy [19]. Another study reported that combined chemotherapy with decitabine and vorinostat could sensitize the tumor cell to Fas-mediated apoptosis and CTL immunotherapy in colon cancer metastasis [20]. Our study indicated that combination treatment with decitabine and PTX causes significant downregulation of LEF1/phospho-β-catenin expression, and the LEF1/β-catenin complex plays a vital role in the synergy of two agents against RCC [21]. The mechanism of action of decitabine in RCC is unclear and should be further investigated.

In this study we analyzed gene-expression profiling and identified specific pathways altered by decitabine in RCC cells. Our results suggest that complex networks and numerous pathways linked to decitabine induce growth inhibition of RCC cells. Moreover, these data confirmed that the p38/NF-κB pathway is involved in G2/M cell cycle arrest of RCC cells induced by decitabine.

Materials and methods

All procedures followed were in accordance with the Ethics Committee of Friendship Hospital, Capital Medical University and with the Helsinki Declaration. Informed consent was obtained from all patients for being included in the study.

Cell culture and agents

Four human ccRCC cell lines (ACHN, Caki-1, Caki-2, and A498) were obtained from the American Type Culture Collection (Manassas, VA, USA), and all RCC cell lines were cultured with complete medium consisting of RPMI-1640 (Gibco, Bio-cult, Glasgow, Scotland), 25 mM HEPES, 1% non-essential amino acids, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 2 mM L-glutamine, and 10% heat-inactivated fetal bovine serum. Cells were maintained as monolayers in 10-cm plastic dishes and incubated at 37°C in a humidified incubator containing 5% CO_2. RCC cells were detached using 0.25% trypsin/0.01% EDTA. Decitabine was purchased from Sigma (St. Louis, MO, USA).

Cell proliferation assay

Cell proliferation was analyzed using the WST-1 assay according to the manufacturer's instructions. RCC cells were planted into 96-well plates and cultured for 24 h, and then the cells were treated with decitabine (1, 2, 4, 8, and 16 μM) for 72 h. After incubation, the cells were treated with 10 μl of WST-1 (Roche, Penzberg, Germany) and the incubation was continued for 2 h. Finally, the absorbance of each well was measured at 450 nm using a microculture plate reader (Immunoreader, Tokyo, Japan). Three independent experiments were performed and untreated cells were used as blank controls.

Flow cytometric analysis

RCC cells were detached using 0.25% trypsin/0.01% EDTA, and 2×10^5 RCC cells were treated with decitabine for 72 h. Cells were collected and fixed with 70% ethanol at -20°C for 8 h, then washed with phosphate-buffered saline and incubated for 30 min with 7-AAD staining solution (BD Biosciences Pharmingen, San Diego, CA, USA). The cells in each phase of the cell cycle were counted with FACSCalibur (Becton Dickinson and analyzed using Cell Quest (version 3.0;).

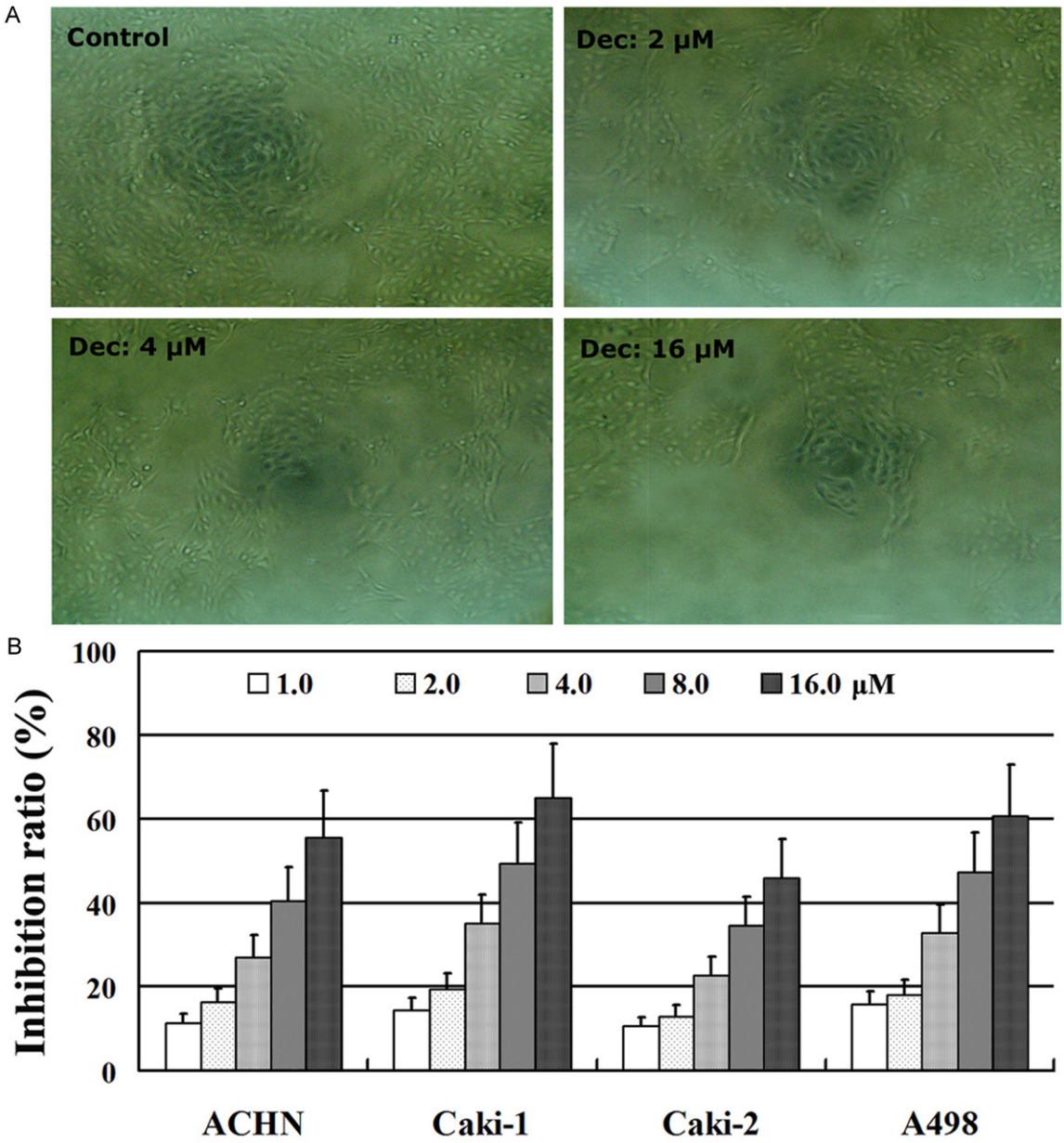

Figure 1. The growth inhibitory effect of decitabine was analyzed in RCC cell lines.

cDNA microarray

Total RNA from the four RCC cell lines treated with decitabine was isolated using Trizol according to the manufacturer's instructions. After RNA measurement and denaturing gel electrophoresis, the RNA samples were amplified and labeled with Cy5 and Cy3 fluorescent dye using the Agilent Quick Amp labeling kit. Hybridizations were performed with Agilent whole genome oligo microarray, and the slides were scanned by the Agilent DNA microarray scanner. We performed sort analysis after pre-treatment to select good spots for scanning or normalization of the array data, and a number of genes potentially relevant to decitabine treatment were identified from our gene array screens.

Western blotting

Western blot was performed according to the manufacturer's instructions. Total protein was isolated from RCC cells treated with decitabine for 72 h. The total protein concentration was measured using a BCA protein assay kit (Pierce, USA). One hundred micrograms of protein was

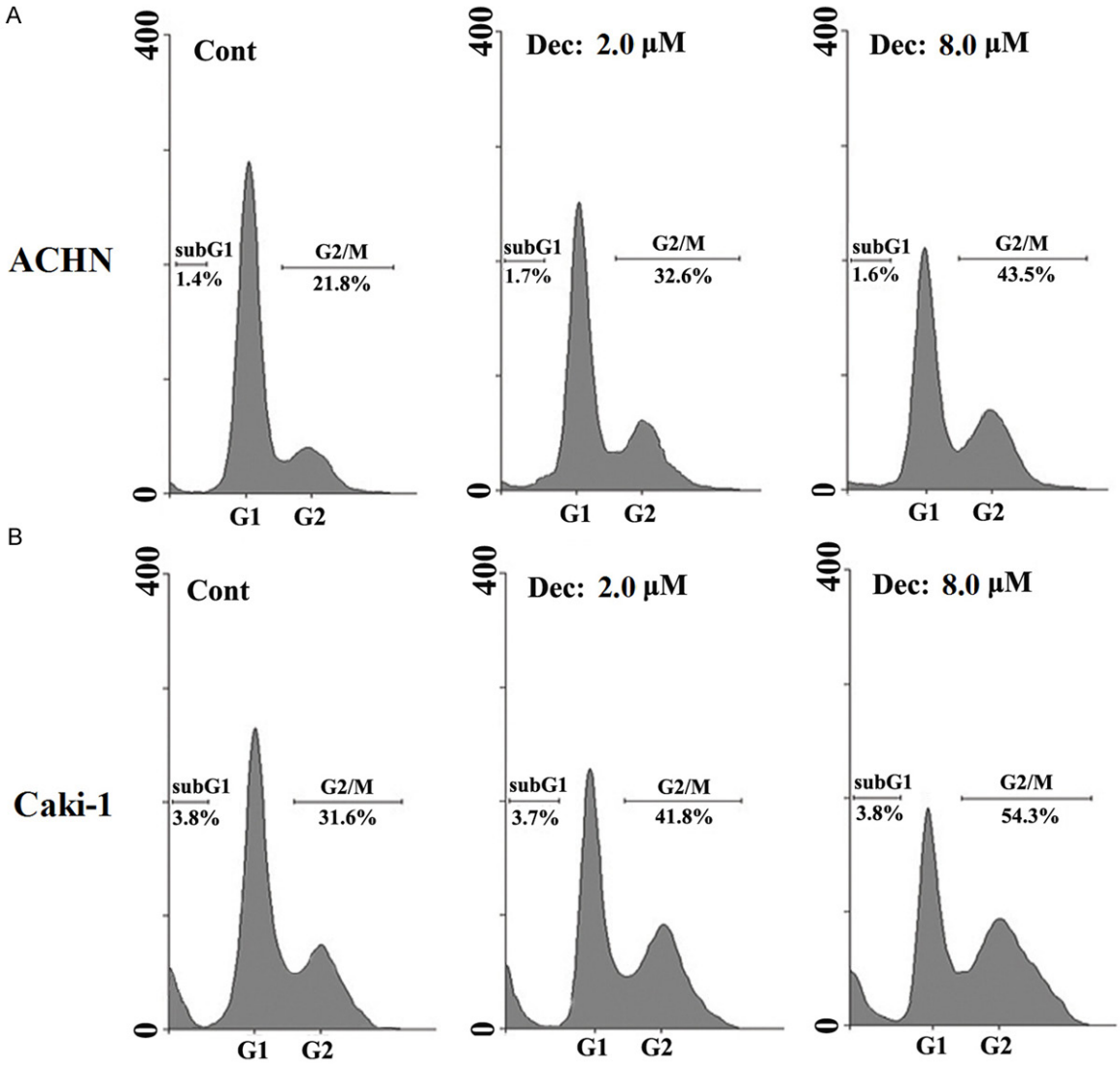

Figure 2. Decitabine did not induce apoptosis in RCC cell lines, rather caused cell cycle arrest in the G2/M phase.

separated by SDS-PAGE and transferred onto PVDF membranes (Amersham, Buckinghamshire, UK). Antibodies against p38 and phospho-p38, NF-κB, and phospho-NF-κB were purchased from Cell Signaling Technology (Danvers, MA, USA). An anti-β-actin monoclonal antibody (Abcam, Cambridge, UK) was applied as a loading control. The immune reaction complexes were visualized with an ECL system (Amersham, Aylesbury, UK).

Statistical analysis

Statistical analysis was determined using SPSS (version 19.0; SPSS, Inc., Chicago, IL, USA). All data were calculated in triplicate and the results are represented as the mean ± standard deviation. Measurement data was investigated using an independent two-tailed t test and a value of $P < 0.05$ was considered statistically significant.

Results

Growth inhibition by decitabine in RCC cells

The growth inhibitory effect of decitabine on RCC cells was analyzed in the four RCC cell lines using the WST-1 assay. RCC cells treated with decitabine had low proliferative ability compared to untreated control cells (**Figure 1A**). Moreover, decitabine inhibited the growth of RCC cells in a dose-dependent manner (**Figure 1B**). Thus, targeting DNA methylation with decitabine may be a novel therapy by which to improve prognosis of patients with advanced RCC.

Table 1. Gene expression regulated by decitabine in RCC cells was investigated using microarray analysis

TOP 10 up-regulated genes by decitabine				
Probe location	Gene bank No.	Gene location	Gene name	Log2 ratio [Decitabine vs. Control]
A_23_P132956	NM_004181	chr4: 40965097..40965156	UCHL1	5.64
A_23_P48513	NM_005532	chr14: 93652682..93652741	IFI27	5.33
A_23_P148541	NM_139250	chrX: 153468202..153468261	CTAG1A	3.91
A_23_P73429	NM_005335	chr3: 122833026..122832969	HCLS1	3.49
A_23_P16523	NM_004864	chr19: 18360890..18360949	GDF15	3.18
A_23_P133408	NM_000758	chr5: 131439351..131439410	CSF2	3.17
A_23_P15542	NM_000413	chr17: 37960565..37960624	HSD17B1	3.16
A_23_P216501	NM_213674	chr9: 35672147..35672088	TPM2	3.14
A_23_P146946	NM_001323	chr11: 65537414..65537473	CST6	2.98
A_23_P76078	NM_016584	chr12: 55020350..55020409	IL23A	2.74
TOP 10 down-regulated genes by decitabine				
Probe location	Gene bank No.	Gene location	Gene name	Log2 ratio [Decitabine vs. Control]
A_24_P916585	AF058296	chr20: 25155371..25155312	ENTPD6	-4.45
A_24_P347458	NM_022474	chr14: 66849057..66849116	MPP5	-3.83
A_24_P323434	NM_152562	chr8: 25393501..25396834	CDCA2	-3.33
A_24_P105283	NM_005066	chr1: 35427247..35426276	SFPQ	-3.8
A_24_P310946	NM_007357	chr1: 228892485..228893774	COG2	-3.72
A_24_P922261	BC029919	chr12: 62761461..62761520	SRGAP1	-3.63
A_23_P84872	NM_024077	chr9: 91163819..91163878	SECISBP2	-3.59
A_23_P76145	AF256215	chr12: 27465419..27465477	ARNTL2	-3.51
A_23_P154217	NM_000888	chr2: 160672479..160666576	ITGB6	-3.47
A_23_P143143	NM_002166	chr2: 8740093..8740481	ID2	-3.36

Apoptotic properties of RCC cells treated by decitabine

We analyzed the induction of apoptosis and cell cycle arrest by decitabine in four RCC cell lines. Flow cytometric analysis indicated that decitabine did not significantly induce apoptosis, even at 8 μM, in all RCC cell lines; however, decitabine caused cell cycle arrest at G2/M in a dose-dependent manner (**Figure 2** [the ACHN and Caki-1 data are shown]).

cDNA microarray analysis

Gene expression regulated by decitabine in RCC cells was investigated using microarray analysis. Top 10 up- or down-regulated genes by decitabine are shown in **Table 1**. The expression of each gene was determined by calculating the average of four RCC cell lines and represented as fold changes compared to untreated control cells. We also selected the possible biological pathways modulated by decitabine in RCC cells using Ingenuity Pathway Analysis (IPA, version 3.0; **Table 2**). The probable pathways induced by decitabine in RCC cells were ranked following the *P* value; a low *P* value represents that the pathway is closely correlated with the effect of decitabine against RCC cells. Our results indicated that the NF-κB pathway, hypoxia, and p53 in the cardiovascular system may be involved in anti-proliferation of decitabine in RCC cells.

Suppression of p38-NF-κB activity by decitabine in RCC cells

To demonstrate that the NF-κB signaling pathway is associated with the effect of decitabine on RCC cells, the activity of the p38-NF-κB signaling pathway was investigated after stimulation with decitabine. In four RCC cell lines, although decitabine did not regulate the total expression of p38 or NF-κB, it suppressed the phosphorylation of p38 and NF-κB (**Figure 3**).

Table 2. Pathways modulated by decitabine in RCC cells were selected by Ingenuity pathway analysis

TOP 10 *pathways modulated by decitabine*	Genes in *the* pathway	Genes in *the* chip	Matched with microarray	*P value*
NF-κB pathway	214	191	19	0.008
Hypoxia and p53 in the cardiovascular system	27	16	4	0.009
amb2 integrin signaling	56	20	5	0.012
TRAIL signaling pathway	633	246	20	0.013
Class I PI3K signaling events	449	181	15	0.02
PDGF signaling	45	35	5	0.025
IL2-mediated signaling events	179	64	9	0.026
eNOS activity regulation	27	7	2	0.035
mTORC1-mediated signaling	12	7	2	0.035
IL23-mediated signaling events	99	40	5	0.036

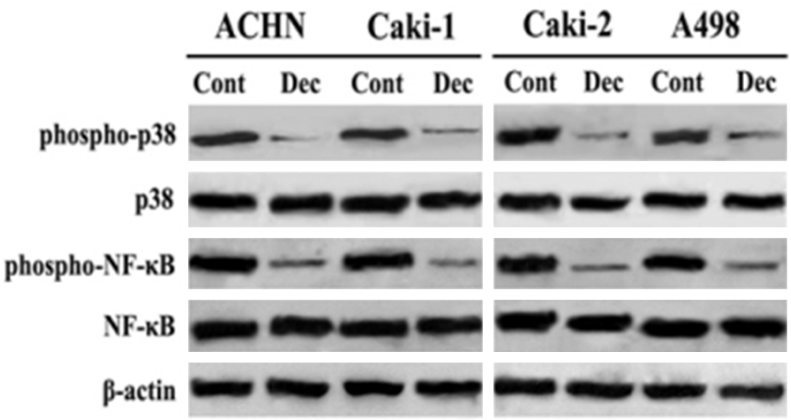

Figure 3. Decitabine suppressed the phosphorylation of p38 and NF-κB.

These results suggest that the p38-NF-κB signaling pathway was suppressed by decitabine and p38-NF-κB may play a vital role in the action of decitabine against RCC cells.

Discussion

The prognosis of RCC is poor because most patients are at an advanced stage at the time of diagnosis and current treatments are not effective [22]. Thus, finding new anti-neoplastic drugs for RCC therapy has the potential to improve the clinical strategy and outcomes in patients with RCC. Decitabine, a DNA methyltransferase inhibitor, has been shown to have anti-tumor activities in human carcinoma [23]. Clinical trials with decitabine have also been conducted in patients with RCC [24, 25]; however, the mechanism of action of decitabine against RCC is not entirely clear.

In this study we investigated the effect of decitabine on the growth of RCC cells and identified specific pathways altered by decitabine in RCC cells. Our results indicated that decitabine inhibits the proliferation of RCC cells. Even though decitabine cannot significantly induce apoptosis in RCC cells, decitabine causes cell cycle arrest at G2/M in a dose-dependent manner. Gene expression regulated by decitabine in RCC cells was also analyzed using microarray analysis. Several up- or down-regulated genes were identified and will be investigated in the future. Our results indicated that ubiquitin carboxyl terminal hydrolase (UCHL) 1 and interferon inducible protein (IFI) 27 are significantly up-regulated by decitabine in RCC cells. Although UCHL1 may contribute to colorectal cancer progression by activating the β-catenin/TCF pathway [26], UCHL1 has been reported as a tumor suppressor and promoter methylation has been detected in ovarian and prostate cancers [27, 28]. Aberrant CpG methylation of the UCHL1 promoter is also significantly associated with transcriptional silencing in breast cancer tissues [29]. Interferons (IFNs) manifest their functions by regulating the expression of target genes, knownas IFN-stimulated genes (ISGs). IFI27 (ISG12) is commonly induced by IFNs and sensitizes cells to apoptotic stimuli by mitochondrial membrane destabilization [30]. IFI27 has been reported to have low expression in leiomyomas and plays a role in the IFN pathway during leiomyoma development [31].

Another study indicated that IFI27 is involved in the proliferation of skin keratinocytes and that IFI27 might be a new target for development of a novel anti-psoriasis therapy [32]. Our results also indicated that the expression of cell division cycle-associated (CDCA) 2 is significantly down-regulated by decitabine in RCC cells. CDCA2 plays a critical role in the DNA damage response and regulates the activation of p53. A recent study indicated that the expression of CDCA2 is over-expressed in oral squamous cell carcinoma and is associated with tumor progression [33]. Low expression of CDCA2 can suppress proliferation in the G1 phase [33]; however, the expression of UCHL1, IFI27, and CDCA2 in RCC is unclear, thus, the role of these genes in RCC and growth inhibition induced by decitabine should be further investigated.

In this study we further investigated the biological pathways regulated by decitabine in RCC cells using IPA analysis. Our results indicated that the NF-κB pathway is suppressed significantly and is involved in the anti-proliferative effect of decitabine on RCC cells. To evaluate the role of the NF-κB pathway in RCC cells treated with decitabine, the activity of the p38-NF-κB signaling pathway was analyzed after stimulation with decitabine. Although decitabine cannot modulate the expression of p38 or NF-κB, it decreased the phosphorylation of p38 and NF-κB. Our results suggest that the p38-NF-κB pathway is suppressed by decitabine and p38-NF-κB may play a critical role in the anti-proliferative effect of decitabine against RCC cells.

In conclusion, our study demonstrated that decitabine suppresses the proliferation of RCC cells via G2/M cell cycle arrest by suppressing p38-NF-κB activity. Moreover, targeting DNA methylation with decitabine may be a useful approach for improving prognosis in patients with advanced RCC.

Acknowledgements

Our research was supported by a grant from National Natural Science Foundation of China (81572502) and Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support, code: ZYLX201604.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuting Liu, Department of Pathology, Capital Medical University, Beijing 100069, China. Tel: 86-10-6313-8553; Fax: 86-10-6313-8553; E-mail: yutingliu889@163.com

References

[1] Arai E, Kanai Y. Genetic and epigenetic alterations during renal carcinogenesis. Int J Clin Exp Pathol 2010; 4: 58-73.

[2] Gupta K, Miller JD, Li JZ, Russell MW, Charbonneau C. Epidemio-logic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev 2008; 34: 193-205.

[3] Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet 2009; 373: 1119-1132.

[4] Joseph RW, Kapur P, Serie DJ, Eckel-Passow JE, Parasramka M, Ho T, Cheville JC, Frenkel E, Rakheja D, Brugarolas J, Parker A. Loss of BAP1 protein expression is an independent marker of poor prognosis in patients with low-risk clear cell renal cell carcinoma. Cancer 2013; 120: 1059-1067.

[5] Stewart GD, O'Mahony FC, Powles T, Riddick AC, Harrison DJ, Faratian D. What can molecular pathology contribute to the management of renal cell carcinoma? Nat Rev Urol 2011; 8: 255-265.

[6] Chalitchagorn K, Shuangshoti S, Hourpai N, Kongruttanachok N, Tangkijvanich P, Thongngam D, Voravud N, Sriuranpong V, Mutirangura A. Distinctive pattern of LINE-1 methylation level in normal tissues and the association with carcinogenesis. Oncogene 2004; 23: 8841-8846.

[7] Banks RE, Tirukonda P, Taylor C, Hornigold N, Astuti D, Cohen D, Maher ER, Stanley AJ, Harnden P, Joyce A, Knowles M, Selby PJ. Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer. Cancer Res 2006; 66: 2000-2011.

[8] Morris MR, Hesson LB, Wagner KJ, Morgan NV, Astuti D, Lees RD, Cooper WN, Lee J, Gentle D, Macdonald F, Kishida T, Grundy R, Yao M, Latif F, Maher ER. Multigene methylation analysis of Wilms' tumour and adult renal cell carcinoma. Oncogene 2003; 22: 6794-6801.

[9] Suh I, Weng J, Fernandez-Ranvier G, Shen WT, Duh QY, Clark OH, Kebebew E. Antineoplastic effects of decitabine, an inhibitor of DNA promoter methylation, in adrenocortical carcinoma cells. Arch Surg 2010; 145: 226-232.

[10] Viet CT, Dang D, Achdjian S, Ye Y, Katz SG, Schmidt BL. Decitabine rescues cisplatin resistance in head and neck squamous cell carcinoma. PLoS One 2014 12; 9: e112880.

[11] Lou YF, Zou ZZ, Chen PJ, Huang GB, Li B, Zheng DQ, Yu XR, Luo XY. Combination of gefitinib and

DNA methylation inhibitor decitabine exerts synergistic anti-cancer activity in colon cancer cells. PLoS One 2014; 9: e97719.
[12] Garrido-Laguna I, McGregor KA, Wade M, Weis J, Gilcrease W, Burr L, Soldi R, Jakubowski L, Davidson C, Morrell G, Olpin JD, Boucher K, Jones D, Sharma S. A phase I/II study of decitabine in combination with panitumumab in patients with wild-type (wt) KRAS metastatic colorectal cancer. Invest New Drugs 2013; 31: 1257-1264.
[13] Shang D, Ito N, Kamoto T, Ogawa O. Demethylating agent 5-aza-2'-deoxycytidine enhances susceptibility of renal cell carcinoma to paclitaxel. Urology 2007; 69: 1007-1012.
[14] Shang D, Liu Y, Liu Q, Zhang F, Feng L, Lv W, Tian Y. Synergy of 5-aza-2'-deoxycytidine (DAC) and paclitaxel in both androgen-dependent and -independent prostate cancer cell lines. Cancer Letters 2009; 278: 82-87.
[15] Shang D, Liu Y, Matsui Y, Ito N, Ogawa O. Demethylaing agent 5-aza-2'-deoxycytidine enhances susceptibility of bladder transitional cell carcinoma to cisplatin. Urology 2008; 71: 1220-1225.
[16] Shi H, Wei SH, Leu YW, Rahmatpanah F, Liu JC, Yan PS, Nephew KP, Huang TH. Triple analysis of the cancer epigenome: an integrated microarray system for assessing gene expression, DNA methylation, and histone acetylation. Cancer Res 2003; 63: 2164-2171.
[17] Alleman WG, Tabios RL, Chandramouli GV, Aprelikova ON, Torres-Cabala C, Mendoza A, Rogers C, Sopko NA, Linehan WM, Vasselli JR. The in vitro and in vivo effects of re-expressing methylated von Hippel-Lindau tumor suppressor gene in clear cell renal carcinoma with 5-aza-2'-deoxycytidine. Clin Cancer Res 2004; 10: 7011-7021.
[18] Zhu WG, Hileman T, Ke Y, Wang P, Lu S, Duan W, Dai Z, Tong T, Villalona-Calero MA, Plass C, Otterson GA. 5-aza-2'-deoxycytidine activates the p53/p21Waf1/Cip1 pathway to inhibit cell proliferation. J Biol Chem 2004; 279: 15161-15166.
[19] Wang LX, Mei ZY, Zhou JH, Yao YS, Li YH, Xu YH, Li JX, Gao XN, Zhou MH, Jiang MM, Gao L, Ding Y, Lu XC, Shi JL, Luo XF, Wang J, Wang LL, Qu C, Bai XF, Yu L. Low dose decitabine treatment induces CD80 expression in cancer cells and stimulates tumor specific cytotoxic T lymphocyte responses. PLoS One 2013; 8: e62924.
[20] Yang D, Torres CM, Bardhan K, Zimmerman M, McGaha TL, Liu K. Decitabine and vorinostat cooperate to sensitize colon carcinoma cells to Fas ligand-induced apoptosis in vitro and tumor suppression in vivo. J Immunol 2012; 188: 4441-4449.
[21] Shang D, Liu Y, Xu X, Han T, Tian Y. 5-aza-2'-deoxycytidine enhances susceptibility of renal cell carcinoma to paclitaxel by decreasing LEF1/phospho-β-catenin expression. Cancer Letters 2011; 311: 230-236.
[22] Escudier B, Eisen T, Porta C, Patard JJ, Khoo V, Algaba F, Mulders P, Kataja V; ESMO Guidelines Working Group. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7: vii65-71.
[23] Shin DY, Kim GY, Kim CG, Kim WJ, Kang HS, Choi YH. Anti-invasive effects of decitabine, a DNA methyltransferase inhibitor, through tightening of tight junctions and inhibition of matrix metalloproteinase activities in AGS human gastric carcinoma cells. Oncol Rep 2012; 28: 1043-1050.
[24] Gollob JA, Sciambi CJ, Peterson BL, Richmond T, Thoreson M, Moran K, Dressman HK, Jelinek J, Issa JP. Phase I trial of sequential low-dose 5-aza-2'-deoxycytidine plus high-dose intravenous bolus interleukin-2 in patients with melanoma or renal cell carcinoma. Clin Cancer Res 2006; 12: 4619-4627.
[25] Abele R, Clavel M, Dodion P, Bruntsch U, Gundersen S, Smyth J, Renard J, van Glabbeke M, Pinedo HM. The EORTC Early Clinical Trials Cooperative Group experience with 5-aza-2'-deoxycytidine (NSC 127716) in patients with colo-rectal, head and neck, renal carcinomas and malignant melanomas. Eur J Cancer Clin Oncol 1987; 23: 1921-1924.
[26] Zhong J, Zhao M, Ma Y, Luo Q, Liu J, Wang J, Yuan X, Sang J, Huang C. UCHL1 acts as a colorectal cancer oncogene via activation of the β-catenin/TCF pathway through its deubiquitinating activity. Int J Mol Med 2012; 30: 430-436.
[27] Jin C, Yu W, Lou X, Zhou F, Han X, Zhao N, Lin B. UCHL1 Is a Putative Tumor Suppressor in Ovarian Cancer Cells and Contributes to Cisplatin Resistance. J Cancer 2013; 4: 662-670.
[28] Ummanni R, Jost E, Braig M, Lohmann F, Mundt F, Barett C, Schlomm T, Sauter G, Senff T, Bokemeyer C, Sültmann H, Meyer-Schwesinger C, Brümmendorf TH, Balabanov S. Ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) is a potential tumour suppressor in prostate cancer and is frequently silenced by promoter methylation. Mol Cancer 2011; 10: 129.
[29] Trifa F, Karray-Chouayekh S, Jmaa ZB, Jmal E, Khabir A, Sellami-Boudawara T, Frikha M, Daoud J, Mokdad-Gargouri R. Frequent CpG methylation of ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) in sporadic and hereditary Tunisian breast cancer patients: clinical significance. Med Oncol 2013; 30: 418.
[30] Cheriyath V, Leaman DW, Borden EC. Emerging roles of FAM14 family members (G1P3/ISG

6-16 and ISG12/IFI27) in innate immunity and cancer. J Interferon Cytokine Res 2011; 31: 173-181.

[31] Mihalich A, Viganò P, Gentilini D, Borghi MO, Vignali M, Busacca M, Di Blasio A. Interferon-inducible genes, TNF-related apoptosis-inducing ligand (TRAIL) and interferon inducible protein 27 (IFI27) are negatively regulated in leiomyomas: implications for a role of the interferon pathway in leiomyoma development. Gynecol Endocrinol 2012; 28: 216-219.

[32] Hsieh WL, Huang YH, Wang TM, Ming YC, Tsai CN, Pang JH. IFI27, a novel epidermal growth factor-stabilized protein, is functionally involved in proliferation and cell cycling of human epidermal keratinocytes. Cell Prolif 2015; 48: 187-197.

[33] Uchida F, Uzawa K, Kasamatsu A, Takatori H, Sakamoto Y, Ogawara K, Shiiba M, Bukawa H, Tanzawa H. Overexpression of CDCA2 in human squamous cell carcinoma: correlation with prevention of G1 phase arrest and apoptosis. PLoS One 2013; 8: e56381.