Int J Clin Exp Pathol 2016;9(9):9364-9371
www.ijcep.com /ISSN:1936-2625/IJCEP0033693

Original Article

Correlation between HDAC6 expression and clinical features of renal cell carcinoma

Jun Wang[1], Weixin Yan[1], Jitong Li[2], Shubin Peng[1], Jun Li[1], Hengjun Xiao[1], Xingqiao Wen[3]

[1]Department of Urology, The Third Affiliated Hospital of Sun Yat Sen University, Guangzhou, Guangdong, PR China; [2]The First Clinical Medicine College, Southern Medical University, Guangzhou, Guangdong, PR China; [3]Department of Urology, Shenzhen Hospital of Southern Medical University, Shenzhen, Guangdong, PR China

Received June 14 2016; Accepted July 12, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Background: Currently, the expression of HDAC6 in renal cell carcinoma has not yet been reported. Only little is known about the relationship between HDAC6 and clinical features of renal cell carcinoma as well. This study is aimed to verify the correlation between the expression of HDAC6 and clinical features of the patients with renal cell carcinoma. Methods: 90 patients with renal cell carcinoma were engaged in the study. The renal cell carcinoma tissues and the neighbored normal tissues were collected. By immunohistochemistry staining and tissue microarray, the expression of HDAC6 in both tissues was assessed. Results: HDAC6 in renal cell carcinoma tissue was showed significant lower expression than that in the neighbored normal tissues ($P < 0.05$). The Spearman's correlation assay proved that the expression of HDAC6 in renal cell carcinoma tissue was significantly positive related to T stage and AJCC clinical stages in renal cell tissues ($r > 0$, $P < 0.05$), while that in neighbored normal tissue was significantly negative related to pathological stages, T stage and AJCC clinical stages ($r < 0$, $P < 0.05$). Kaplan-Meier assay and log-rank test proved that high expression of HDAC6 in renal cell carcinoma tissue induced lower survival rate ($P < 0.05$), but that in neighbored normal tissue induced higher survival rate ($P = 0.052$). Conclusion: HDAC6 might perform different functions in renal cell carcinoma tissue and neighbored normal tissue; it might promote tumor angiogenesis in renal cell carcinoma tissue but suppress the tumorigenesis in the normal kidney tissue.

Keywords: HDAC6, renal cell carcinoma, clinic pathological parameters, prognosis, survival

Introduction

Renal cell carcinoma (abbreviated as renal carcinoma) is a malignant cancer that affects human's health. It accounts for 3% of adult malignancies and reach up to 90% of renal tumors [1]. The potential root of the renal cell carcinoma remains unclear and it can recur many years after treatment. As renal cell carcinoma has poor response to chemotherapy, radiotherapy and surgery [2], early diagnoses are important for patients [3, 4]. The five-year survival rate for the patients without metastasis, the survival can reach 90%. However, it can be reduced to less than 10% once the metastasis occurs [5]. The common sites of the renal cell carcinoma can be lungs, lymph nodes, bones and brains [6-8]. However, renal cell carcinoma can also metastasize to many unusual sites and affect most of the organs, such as pancreas, skin or some soft tissues [9]. Currently, the clinical and pathological parameters are widely used for RCC prognosis, including TNM stage, Fuhrman nuclear grade, ECOG performance status, tumor necrosis, etc. Some integrated prognostic models based on these factors have been established for the prediction of patients' clinical outcome, such as University of California Integrated Staging System (UISS) and Mayo Clinic stage, size, grade and necrosis score (SSIGN) [10]. Since renal cell carcinoma is complicated disease, those models cannot be always accurate. Moreover, the clinical significance of some molecular biological markers is underestimated at most time. Therefore, better and comprehensive understanding of the molecular mechanism of the occurrence, development and also metastasis of renal cell carcinoma can help early diagnosis to the diseases and benefits to the current prognostic models.

Histone deacetylases (HDACs) are classified into four groups, including the class I (HGAC1-3

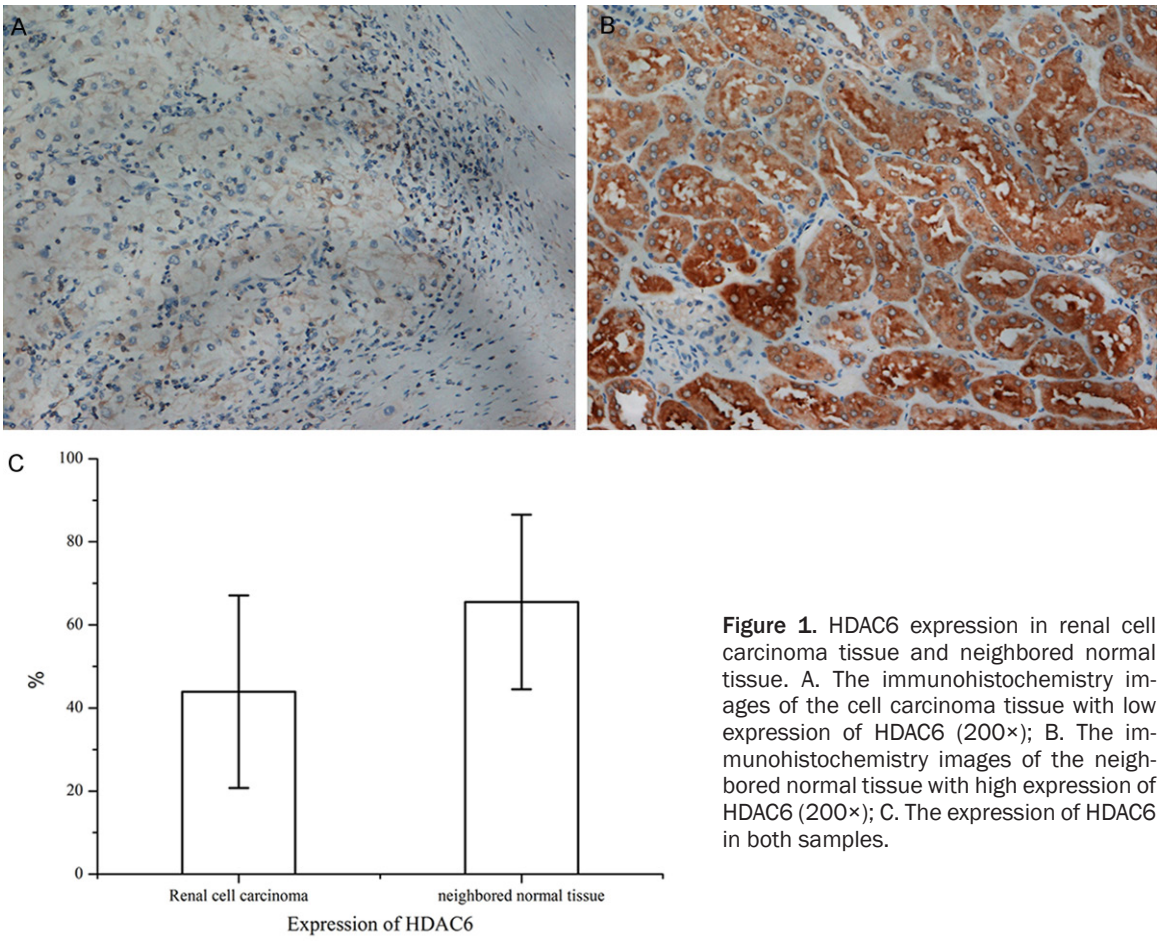

Figure 1. HDAC6 expression in renal cell carcinoma tissue and neighbored normal tissue. A. The immunohistochemistry images of the cell carcinoma tissue with low expression of HDAC6 (200×); B. The immunohistochemistry images of the neighbored normal tissue with high expression of HDAC6 (200×); C. The expression of HDAC6 in both samples.

and 8), class II (HDAC4-7 and 9-10), class III (SIRT1-7) and class IV (HDAC11) [11-14]. HDAC6 is the member of HDACs and belongs to the class II histone deacetylase/acuc/apha family [15]. Previous studies demonstrated that it plays crucial role in various cancers by performing regulation in cell cycle progression, autophagy and apoptosis [16, 17]. Therefore, HDAC6 might be closely related with the occurrence and development of tumor. Besides, the high expression of HDAC6 has been found in glioma, breast cancer, prostate cancer and other tumors [18-20]. However, there are also some reports suggested that the HDAC6 shows low expression in hepatocellular carcinoma which indicates its function as tumor suppressor [21]. Currently, the expression of HDAC6 in renal cell carcinoma has not yet been reported. Only little is known about the relationship between HDAC6 and clinical features of renal cell carcinoma as well.

In this study, the tumor tissues of renal carcinoma from 90 clinical diagnosed patients were collected via surgery. And then followed by the tissue microarray and immunohistochemistry stain for detecting the expression of HDAC6. Prognosis analysis between expression of HDAC6 and survival rate of the patients was carried out. We aimed to evaluate the clinical pathologic and prognostic significance of HDAC6 expression in patients with renal cell carcinoma.

Materials and methods

Ethics statement

The present study was approved by the Ethics Committee of The Third Affiliated Hospital of Sun-Yat Sen University and was conducted in accordance with the Declaration of Helsinki Principles. All participants provided signed informed written consent in advance.

Tissue samples collection

Kidney tissue samples for this study were obtained from 90 patients who underwent radi-

Table 1. Association between HDAC6 and clinical parameters of patients

Clinical parameter	HDAC6 expression		Total	χ^2	*P*
	Low	High			
Gender				0.436	0.509
Male	20	31	51		
Female	18	21	39		
Age				0.981	0.322
≤60	23	26	49		
>60	15	26	41		
Size				0.454	0.501
≤5 cm	21	25	46		
>5 cm	17	27	44		
Fuhrman grade				3.855	0.278
I	15	18	33		
II	20	22	42		
III	3	11	14		
IV	0	1	1		
Distant metastasis				1.495	0.221
No	38	50	88		
Yes	0	2	2		
T stage				8.169	0.086
T1a	13	20	33		
T1b	18	12	30		
T2a	5	10	15		
T2b	1	2	3		
T3	0	5	5		
N stage				0.7	0.403
N0	36	51	87		
N1	0	1	1		
M stage				1.495	0.221
M0	38	50	88		
M1	0	2	2		
AJCC Clinical stage				5.897	0.117
1	29	31	60		
2	6	12	18		
3	0	4	4		
4	0	2	2		

cal or partial nephrectomy for RCC and diagnosed at The Third Affiliated Hospital of Sun-Yat Sen University from July 2004 to February 2006. The samples were collected from normal tumor neighbored tissues (outside from the tumor at least 5 cm) and tumor tissues from each patient. Samples were immediately fixed in 10% formalin solution for 14 hours. Then paraffin embedment was performed for further immunohistochemically study. Pathological stage was determined by Fuhrman nuclear grade. Tumor stage was determined according to the 2010 TNM classification of American Joint Committee on Cancer (AJCC). Prognosis assay was done in order to follow-up with survivors and estimate the overall survival rate. Median follow-up was 99 months (range: 84-114 months). OS was calculated from the date of surgery to the date of death or to the date of the latest follow-up.

Tissue array and immunohistochemistry staining

The human renal cell cancer tissue microarray was constructed with formalin-fixed and paraffin-embedded kidney tissues and with the help of Outdo Biotech (Shanghai, China). Immunohistochemically studies were performed using the Leica autostainer XL ST5010 (Leica Biosystems, Wetzlar, Germany). Briefly, the sections were deparaffinized in xylene and dehydrated through ethanol, and then subjected to antigen retrieval in citrate buffer (10 mM, pH 6.0) for 30 min. Next, the sections were blocked through bath slides in Peroxidase-Blocking Reagent (Dako Cytomation, Glostrup, Denmark) for 15 minutes at room temperature, followed by incubation with rabbit anti-human polyclonal HDAC6 primary antibody (1:1000 dilution, Abcam, Cambridge, UK) for overnight at 4°C in a humidified chamber. Sections were then incubated with the mouse anti-rabbit secondary antibody (1:1500 dilution, Abcam, Cambridge, UK) for 30 min at room temperature. After a complete wash in PBS, the sections were developed in freshly prepared diaminobenzidine solution (DAB) then counterstained with hematoxylin, dehydrated through graded ethanol, cleared with xylene, and cover-slipped. The expression of HDAC6 was estimated according to the percentage of stained cells. In each sample, it was considered as either low-expression (≤ 30%) or high-expression (> 30%).

Statistical analysis

Association of the expression of HDAC6 and pathological parameters was calculated by Chi-square test. The expression of HDAC6 between normal samples and renal cell carcinoma sam-

Table 2. Spearman's correlation assay of the correlation between HDAC6 expression and clinical parameters in two groups

		Gender	Age	Size	Fuhrman grade	T	N	M	AJCC Clinical stage
Renal cell carcinoma tissue	Correlation Coefficient	-0.055	0.094	0.086	0.133	0.218	0.088	0.128	0.228
	Sig. (2-tailed)	0.606	0.383	0.425	0.214	0.045	0.415	0.232	0.038
	N	89	89	89	89	85	87	89	83
Neighbored normal tissue	Correlation Coefficient	-0.13	-0.033	-0.168	-0.24	-0.351	0.039	-0.186	-0.329
	Sig. (2-tailed)	0.227	0.762	0.118	0.024	0.001	0.719	0.083	0.003
	N	88	88	88	88	84	86	88	82

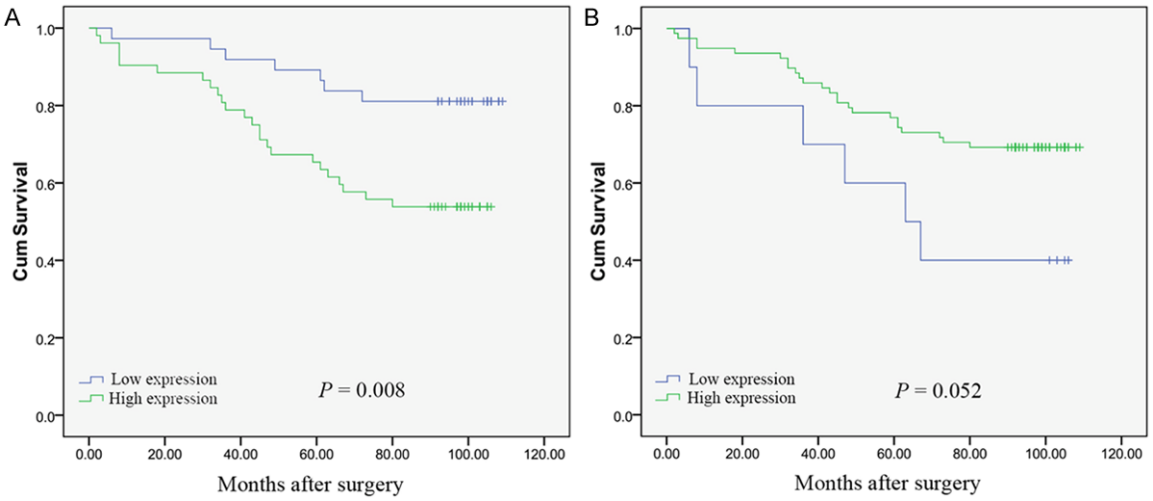

Figure 2. Overall survival analysis of patients based on HDAC6 expression in renal cell carcinoma tissue and neighbored normal tissue. Kaplan-Meier analysis of overall survival related to the HDAC6 expression in renal cell carcinoma tissue (A). And neighbored normal tissue (B). *P* value was calculated by log-rank test.

ples was compared using student two-tailed t-test. The correlation between these two groups of samples was evaluated using Pearson correlation test. The correlation between the expression of HDAC6 and pathologic indexes of the patients was calculated using Spearman's correlation analysis.

The Kaplan-Meier method was used to analyze patient survival, and the log-rank test was used to estimate the differences between groups. Overall survival was defined as the interval from the date of initial surgery to the date of death or last contact. The Cox proportional hazards regression model was performed for multivariate analysis to study the contribution of various potential prognostic parameters to overall survival. All the data were presented as mean value ± standard deviation. The significance was established as $P < 0.05$. All the analyses were performed using SPSS 19.0 software.

Results

Detection of HDAC6 expression by immunohistochemistry staining and the clinic pathological parameters

The results of the immunohistochemistry staining showed that HDAC6 was predominantly localized in the membrane and cytoplasm of both renal cancer cells and normal renal cells. Indeed, HDAC6 was expressed in 99% (89/90) of all tumor samples, whereas it was expressed in 98% (88/90) of normal neighbored tissue samples. HDAC6 presented lower expression in the renal cancer cells (43.91%±23.20%) compared with the normal renal cells (65.2%± 21.01%). It is also significantly presented that the normal cells with high expression of HDAC6 can be observed widespread brown color, whereas the HDAC6 was hardly stained in the renal cell tissues (**Figure 1**). However, no significant difference was found between the expres-

Table 3. Multivariate Cox regression analysis of overall survival

	B	SE	Wald	df	*P*-value	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
HDAC6 expression	0.664	0.448	2.192	1	0.139	1.942	0.807	4.677
Age	0.719	0.394	3.322	1	0.068	2.051	0.947	4.442
Fuhrman grade	0.818	0.312	6.876	1	0.009	2.265	1.229	4.174
T	0.677	0.385	3.093	1	0.079	1.967	0.925	4.183
N	2.1	1.199	3.069	1	0.08	8.165	0.779	85.564
M	1.088	0.947	1.321	1	0.25	2.968	0.464	18.978

sion of HDAC6 and clinical parameters (gender, age, size, distant metastasis, Fuhrman grade, TNM stages and AJCC clinical stage) (**Table 1**).

Correlation between expression of HDAC6 in renal cell and normal tissue and pathological parameters

The Pearson correlation assay showed that for the expression of HDAC6 in renal cell cancer tissue and normal neighbor tissue are not significant ($P = 0.625$). However, further analysis done by Spearman's correlation assay showed that the expression of HDAC6 is significantly positive related to T stage and AJCC clinical stages in renal cell tissues ($r > 0$, $P < 0.05$), while significantly negative related to pathological stages, T stage and AJCC clinical stages in normal neighbor tissue ($r < 0$, $P < 0.05$) (**Table 2**). In another word, HDAC6 might be functionally related to renal cell, but the functions of HDAC6 might be different between renal cell carcinoma tissue and normal kidney tissue.

Correlation between expression of HDAC6 and patient's prognosis

In the overview, the overall survival rate is around 65.2%. According to the results from Kaplan-Meier assay and log-rank test, it represents that the survival rate of patients who tended to be high HDAC6-expressed in renal cell cancer tissues was significant lower than that of the low HDAC6-expressed patients ($P < 0.05$) (**Figure 2A**). The overall survival rate for high HDAC6-expression group is 53.8% (28/52), while for the low HDAC6-expression group is 81.1% (30/37). However, for those patients with high HDAC6-expression in normal neighbored tissue showed higher survival rate than those with low HDAC6-expression (**Figure 2B**). The overall survival rate of the group with high HDAC6-expression in normal neighbored tissue is 69.2% (54/78) and that with low HDAC6-expression is 40% (4/10), but the result is not significant ($P = 0.052$).

For further verification, Cox proportional hazards regression model was performed (**Table 3**). The results are controversial to the Kaplan-Meier assay and log-rank test that HDAC6 expression is not the significant prognostic factor for patients with renal cell cancer ($P > 0.05$). Additionally, age, TNM stages and clinical stages were not significant prognostic factors as well ($P > 0.05$), but only pathological stages showed high significance ($P < 0.05$).

Discussion

According to the previous studies, HDACs is able to repress the transcription of specific genes that improve or suppress the progress of cancer. As a molecular biological target, it has been widely used in development of new drugs or therapies. However, the exact functions of HDAC6 perform to cancer is remained undetermined. For some cases in hepatocellular carcinoma and cholangiocarcinoma, high-expressed HDAC6 will suppress tumor growth by activating autophagic cell death [22-24]. However, there are researches showed that HDAC6 might promote carcinoma angiogenesis in malignant thyroid carcinoma [25] and hepatocellular carcinoma [21]. However, for the functions that HDAC6 performs in renal cell carcinoma, it is still remained unknown. In this study, it is focused on verifying the correlation between the expression of HDAC6 and the prognosis in renal cell carcinoma.

The present results showed that the expression of HDAC6 in renal cell carcinoma samples was higher than that in the normal kidney samples.

The result is consistent with the previous studies from Jung et al. [26] and Lv et al. [21]. The expression of HDAC6 in both tissues is not significantly related. Thus, it can be predicted that, the main functions of HDAC6 perform in renal cell carcinoma tissue and normal kidney tissue can be different. Additionally, the correlation assays for prognosis of patients showed that the expression of HDAC is positively related to T stage and clinical stage in renal cell carcinoma tissue, while is negatively related to T stage, clinical stage and pathological grade in normal neighbored kidney tissue. Therefore, it can be concluded that HDAC6 promote tumor angiogenesis in renal cell carcinoma tissue but suppress the tumor development in the normal kidney tissue. However, the mechanism behind is still unclear. According to the previous studies, HDAC6 is usually regarded as the tumor suppressor since it is linked to c-Jun NH2-terminial kinase and NF-κB signaling pathway that eventually suppress oncogenic tumorigenesis [26, 27]. However, HDAC6 was reported that it could promote the growth of tumor [28]. The regulation is performed via the inhibition of SMAD2 signaling [29]. These inconsistent results indicate that the underlying functional mechanism of HDAC6 should be complicated and various.

For further results from the Kaplan-Meier and log-rank test suggested that patients with high HDAC6 expression showed lower survival rate than those with low HDAC6 expression in renal cell carcinoma tissue. However, it was opposite in neighbored normal kidney tissue that the patients with high HDAC6 expression survived longer than those with low HDAC6 expression. However, Cox proportional hazards regression model proved against the conclusion above that the expression of HDAC6 was not the significant prognosis factor of renal cell carcinoma. The result is consistent with the research focused on breast cancer from Park et al. [30], whereas it is different from the study concerned on prostatic foamy gland carcinoma from Hou et al. that proved HDAC6 is the significant prognostic factor [31]. Since the former research with large volume of 314 samples, the result would be more persuasive than the later one. However, most of the researches provided the common evidences that the expression of HDAC6 should be, to some extent, correlated to the overall survival of patients [30-33].

On the basis of the studies of HDACs, HDAC inhibitors were developed and widely used for clinical purpose. HDAC inhibitors act by modifying gene expression and are currently the newest class of drugs shown to be promising in patients with several malignancies. The results from the researches so far about HDACs-targeting therapy across different cancer cell lines showed the advanced efficiency of HDAC inhibitors. Most of the HDAC inhibitors can significantly suppress tumor angiogenesis and lead to cell apoptosis in such as colorectal cancer [34-36]. For renal cell carcinoma, the combined therapy of HDAC inhibitors, panobinostat and bortezomib can not only significantly suppress the proliferation of renal cell carcinoma cell line and then lead to cell apoptosis, but also inhibit the tumorigenesis [37]. Moreover, the HDACs inhibitor, panobinostat, was entitled and approved by FDA to be used as the treatment for the patients with multiplemyoloam [38-40]. Therefore, comprehensive understanding of HDACs can help with the development of the HDACs targeting therapies and will eventually benefits to human health. Although the cytological researches provided theoretic insight of the HDAC inhibitors for clinical application, the exact functions that HDAC6 performs in different tissues needs to be further identified. In this study, HDAC6 was found to perform controversial functions respectively in renal cell carcinoma and neighbored normal tissue. However, the exact regulation network and the key functionally related genes were remained uncertain. In the future study, for further understanding the regulation mechanism of HDAC6, the focus will not only be restricted to various renal cell carcinoma cell lines, the functions of HDAC6 in normal renal tubular epithelial cell and renal tubular epithelial cell metaplasia needs to be investigated as well.

In conclusions, HDAC6 might perform different functions in renal cell carcinoma tissue and neighbored normal tissue; it might promote tumor angiogenesis in renal cell carcinoma tissue but suppress the tumorigenesis in the normal kidney tissue.

Acknowledgements

This work was supported by Science and Technology Planning Project of Guangdong Province (2014A020212063).

Disclosure of conflict of interest

None.

Address correspondence to: Hengjun Xiao, Department of Urology, The Third Affiliated Hospital of Sun Yat Sen University, 600 Tianhe Road, Tianhe District, Guangzhou 510630, PR China. E-mail: hjxiao555@126.com; Xingqiao Wen, Department of Urology, Shenzhen Hospital of Southern Medical University, 1333 Xinhu Road, Baoan District, Shenzhen 518100, PR China. Tel: +86-20-85253080; E-mail: wenxingqiaoszhos@163.com

References

[1] Garcia JA, Cowey CL, Godley PA. Renal cell carcinoma. Curr Opin Oncol 2009; 21: 266-271.
[2] Doh LS, Amato RJ, Paulino AC, Teh BS. Radiation therapy in the management of brain metastases from renal cell carcinoma. Oncology 2006; 20: 603-613; discussion 613, 616, 619-620 passsim.
[3] Breau RH, Blute ML. Surgery for renal cell carcinoma metastases. Curr Opin Urol 2010; 20: 375-381.
[4] Saitoh H, Nakayama M, Nakamura K, Satoh T. Distant metastasis of renal adenocarcinoma in nephrectomized cases. J Urol 1982; 127: 1092-1095.
[5] Drucker BJ. Renal cell carcinoma: current status and future prospects. Cancer Treat Rev 2005; 31: 536-545.
[6] Flanigan RC, Campbell SC, Clark JI, Picken MM. Metastatic renal cell carcinoma. Curr Treat Options Oncol 2003; 4: 385-390.
[7] Palazzo FF, Bradpiece HA, Morgan MW. Renal cell carcinoma metastasizing to the thyroid gland. Scand J Urol Nephrol 1999; 33: 202-204.
[8] van der Poel HG, Roukema JA, Horenblas S, van Geel AN, Debruyne FM. Metastasectomy in renal cell carcinoma: A multicenter retrospective analysis. Eur Urol 1999; 35: 197-203.
[9] Brady LW, O'Neill EA, Farber SH. Unusual sites of metastases. Semin Oncol 1977; 4: 59-64.
[10] Sun M, Shariat SF, Cheng C, Ficarra V, Murai M, Oudard S, Pantuck AJ, Zigeuner R, Karakiewicz PI. Prognostic factors and predictive models in renal cell carcinoma: a contemporary review. Eur Urol 2011; 60: 644-661.
[11] Vigushin DM, Coombes RC. Histone deacetylase inhibitors in cancer treatment. Anti-cancer Drugs 2002; 13: 1-13.
[12] Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. J Natl Cancer Inst 2000; 92: 1210-1216.
[13] Coffey DC, Kutko MC, Glick RD, Swendeman SL, Butler L, Rifkind R, Marks PA, Richon VM, LaQuaglia MP. Histone deacetylase inhibitors and retinoic acids inhibit growth of human neuroblastoma in vitro. Med Pediatr Oncol 2000; 35: 577-581.
[14] Schrump DS. Cytotoxicity mediated by histone deacetylase inhibitors in cancer cells: mechanisms and potential clinical implications. Clin Cancer Res 2009; 15: 3947-3957.
[15] Valenzuela-Fernandez A, Cabrero JR, Serrador JM, Sanchez-Madrid F. HDAC6: a key regulator of cytoskeleton, cell migration and cell-cell interactions. Trends Cell Biol 2008; 18: 291-297.
[16] Ververis K, Hiong A, Karagiannis TC, Licciardi PV. Histone deacetylase inhibitors (HDACIs): multitargeted anticancer agents. Biologics 2013; 7: 47-60.
[17] West AC, Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. J Clin Invest 2014; 124: 30-39.
[18] Wu Y, Song SW, Sun J, Bruner JM, Fuller GN, Zhang W. IIp45 inhibits cell migration through inhibition of HDAC6. J Biol Chem 2010; 285: 3554-3560.
[19] Rey M, Irondelle M, Waharte F, Lizarraga F, Chavrier P. HDAC6 is required for invadopodia activity and invasion by breast tumor cells. Eur J Cell Biol 2011; 90: 128-135.
[20] Ai J, Wang Y, Dar JA, Liu J, Liu L, Nelson JB, Wang Z. HDAC6 regulates androgen receptor hypersensitivity and nuclear localization via modulating Hsp90 acetylation in castration-resistant prostate cancer. Mol Endocrinol 2009; 23: 1963-1972.
[21] Lv Z, Weng X, Du C, Zhang C, Xiao H, Cai X, Ye S, Cheng J, Ding C, Xie H, Zhou L, Wu J, Zheng S. Downregulation of HDAC6 promotes angiogenesis in hepatocellular carcinoma cells and predicts poor prognosis in liver transplantation patients. Mol Carcinog 2016; 55: 1024-1033.
[22] Gradilone SA, Radtke BN, Bogert PS, Huang BQ, Gajdos GB, LaRusso NF. HDAC6 inhibition restores ciliary expression and decreases tumor growth. Cancer Res 2013; 73: 2259-2270.
[23] Ding G, Liu HD, Huang Q, Liang HX, Ding ZH, Liao ZJ, Huang G. HDAC6 promotes hepatocellular carcinoma progression by inhibiting P53 transcriptional activity. FEBS Lett 2013; 587: 880-886.
[24] Kanno K, Kanno S, Nitta H, Uesugi N, Sugai T, Masuda T, Wakabayashi G, Maesawa C. Overexpression of histone deacetylase 6 contributes to accelerated migration and invasion activity of hepatocellular carcinoma cells. Oncol Rep 2012; 28: 867-873.
[25] Giaginis C, Alexandrou P, Delladetsima I, Giannopoulou I, Patsouris E, Theocharis S. Clinical

significance of histone deacetylase (HDAC)-1, HDAC-2, HDAC-4, and HDAC-6 expression in human malignant and benign thyroid lesions. Tumour Biol 2014; 35: 61-71.

[26] Jung KH, Noh JH, Kim JK, Eun JW, Bae HJ, Chang YG, Kim MG, Park WS, Lee JY, Lee SY, Chu IS, Nam SW. Histone deacetylase 6 functions as a tumor suppressor by activating c-Jun NH2-terminal kinase-mediated beclin 1-dependent autophagic cell death in liver cancer. Hepatology 2012; 56: 644-657.

[27] Bae HJ, Jung KH, Eun JW, Shen Q, Kim HS, Park SJ, Shin WC, Yang HD, Park WS, Lee JY, Nam SW. MicroRNA-221 governs tumor suppressor HDAC6 to potentiate malignant progression of liver cancer. J Hepatol 2015; 63: 408-419.

[28] Lee YS, Lim KH, Guo X, Kawaguchi Y, Gao Y, Barrientos T, Ordentlich P, Wang XF, Counter CM, Yao TP. The cytoplasmic deacetylase HDAC6 is required for efficient oncogenic tumorigenesis. Cancer Res 2008; 68: 7561-7569.

[29] Li S, Liu X, Chen X, Zhang L, Wang X. Histone deacetylase 6 promotes growth of glioblastoma through inhibition of SMAD2 signaling. Tumour Biol 2015; 36: 9661-9665.

[30] Park Y, Lee KS, Park SY, Kim JH, Kang EY, Kim SW, Eom KY, Kim JS, Kim IA. Potential Prognostic Value of Histone Deacetylase 6 and Acetylated Heat-Shock Protein 90 in Early-Stage Breast Cancer. J Breast Cancer 2015; 18: 249-255.

[31] Hou H, Zhao L, Chen W, Li J, Zuo Q, Zhang G, Zhang X, Li X. Expression and significance of cortactin and HDAC6 in human prostatic foamy gland carcinoma. Int J Exp Pathol 2015; 96: 248-254.

[32] Zhang Z, Yamashita H, Toyama T, Sugiura H, Omoto Y, Ando Y, Mita K, Hamaguchi M, Hayashi S, Iwase H. HDAC6 expression is correlated with better survival in breast cancer. Clin Cancer Res 2004; 10: 6962-6968.

[33] Saji S, Kawakami M, Hayashi S, Yoshida N, Hirose M, Horiguchi S, Itoh A, Funata N, Schreiber SL, Yoshida M, Toi M. Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer. Oncogene 2005; 24: 4531-4539.

[34] Zhijun H, Shusheng W, Han M, Jianping L, Li-Sen Q, Dechun L. Pre-clinical characterization of 4SC-202, a novel class I HDAC inhibitor, against colorectal cancer cells. Tumour Biol 2016; 37: 10257-67.

[35] Debeb BG, Lacerda L, Xu W, Larson R, Solley T, Atkinson R, Sulman EP, Ueno NT, Krishnamurthy S, Reuben JM, Buchholz TA, Woodward WA. Histone deacetylase inhibitors stimulate dedifferentiation of human breast cancer cells through WNT/beta-catenin signaling. Stem Cells 2012; 30: 2366-2377.

[36] Jaeger M, Ghisleni EC, Fratini L, Brunetto AL, Gregianin LJ, Brunetto AT, Schwartsmann G, de Farias CB, Roesler R. Viability of D283 medulloblastoma cells treated with a histone deacetylase inhibitor combined with bombesin receptor antagonists. Childs Nerv Syst 2016; 32: 61-64.

[37] Sato A, Asano T, Isono M, Ito K, Asano T. Panobinostat synergizes with bortezomib to induce endoplasmic reticulum stress and ubiquitinated protein accumulation in renal cancer cells. BMC Urol 2014; 14: 71.

[38] Yoon S, Eom GH. HDAC and HDAC Inhibitor: From Cancer to Cardiovascular Diseases. Chonnam Med J 2016; 52: 1-11.

[39] Afifi S, Michael A, Azimi M, Rodriguez M, Lendvai N, Landgren O. Role of Histone Deacetylase Inhibitors in Relapsed Refractory Multiple Myeloma: A Focus on Vorinostat and Panobinostat. Pharmacotherapy 2015; 35: 1173-1188.

[40] Laubach JP, Moreau P, San-Miguel JF, Richardson PG. Panobinostat for the Treatment of Multiple Myeloma. Clin Cancer Res 2015; 21: 4767-4773.