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Original Article

SIRT4 is upregulated in Chinese patients with esophageal cancer

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Abstract: Background and objective: Several members of the sirtuin (SIRT) family, a highly conserved family of NAD+-dependent enzymes, may influence tumor formation. SIRT4 regulates glutamine metabolism, but also may act as a tumor suppressor. The current study aimed to investigate whether SIRT4 is associated with occurrence and development of Chinese esophageal squamous cell carcinoma (ESCC). Method: We investigated the SIRT4 protein levels in ESCC and possible association with selected clinicopathological parameters and the overall survival by immunohistochemical staining on a tissue microarray that included 93 ESCC patients. Results: SIRT4 protein levels in ESCC tissues were higher than in corresponding normal tissues. Besides, women esophageal cancer patients are more likely have high levels of SIRT4 protein in ESCC tissues. The average survival time of patients with high SIRT4 levels in esophageal cancer tissues was shorter than that of patients with low SIRT4 levels. Conclusions: Our results indicate that SIRT4 may participate in the development of esophageal cancer.

Keywords: SIRT4, carcinogenesis, esophageal squamous cell carcinoma

Introduction

Esophageal cancer is the sixth leading cause of cancer-related death worldwide. In 2012, there were about 400,200 deaths due to esophageal cancer [1]. The prognosis of patients with esophageal squamous cell carcinoma (ESCC) is poor; the postoperative 5-year survival rate is only about 15% [2]. The etiology and pathogenesis of esophageal cancer is very complex, involving many risk factors and a variety of genetic and epigenetic alterations. Over the past few decades, many key genes and signaling pathways were found involved in the pathogenesis of esophageal cancer. These include tumor suppressor gene TP53, CDKN2A, RTK-MAPK-PI3K and the Notch signaling pathway [3]. To discover potentially useful targets to aid diagnosis and therapeutic strategies, a better understanding is required of the molecular mechanisms that underlie the gene changes and pathology of esophageal carcinoma.

The sirtuin (SIRT) family is a 7-member group (SIRT1-7) of NAD+-dependent acetylases, deacetylases, and ADP-ribosyltransferases that are involved in pressure resistance, genome stability, energy metabolism, and aging [4]. In particular, SIRT4 is an NAD+-dependent ADP-ribosyltransferase located in the mitochondria, which catalyzes and transfer of ADP ribosyl to glutamate dehydrogenase [5]. Recent studies indicate that SIRT4 acts as a tumor suppressor by regulating the metabolism of glutamine [6, 7]. Several studies have also found that SIRT4 levels are downregulated in gastric cancer and colon cancer tissues and are associated with some clinicopathological parameters [8-10]. Recently, there have been reports that downregulation of SIRT4 levels is associated with poor prognosis in ESCC [11]. However, the study did not compare the SIRT4 levels of esophageal cancer tissues with that of adjacent normal tissues. Furthermore, the study was limited by basing evidence solely on the immunohistochemistry of paraffin sections, rather than tissue microarray. To confirm the important results of Nakahara et al. [11], reliable comparisons of the SIRT4 levels between ESCC tissue and adjacent normal tissues should be conducted, as

Table 1. SIRT4 protein expression in hepatocellular carcinoma and adjacent normal liver tissues

			SIRT4 levels			
		All cases	Low	High	χ^2	*P**
Age, y	≤ 60	27	10	17	1.014	0.314
	> 60	66	32	34		
Gender	Male	77	39	38	5.443	**0.026**
	Female	16	3	13		
Tumor size, cm	≤ 5	51	23	28	0.000	0.989
	> 5	42	19	23		
Differentiation	Well	14	3	11	4.281	0.118
	Moderate	60	31	29		
	Poor	19	8	11		
T stage	T1-T2	22	11	11	0.587	0.444
	T3-T4	64	26	38		
N stage	N0	45	23	22	1.246	0.302
	N1-N3	48	19	29		
AJCC stage	I	11	3	8	2.639	0.267
	II	37	19	18		
	III	40	15	25		

Bold values are statistically significant (P < 0.05). *Chi-squared test.

well as investigations of associations between SIRT4 levels and the clinicopathological parameters of ESCC patients.

The present study utilized high-throughput tissue microarray and immunohistochemistry to compare the SIRT4 levels of ESCC tissue and matched normal tissues. We also analyzed the association between SIRT4 levels and clinicopathological parameters in ESCC, and between SIRT4 levels and patient outcomes to determine its value as a prognostic indicator.

Materials and methods

The Ethics Committee of First Affiliated Hospital of Wenzhou Medical University approved this study. The study was performed in accordance with the principles of the Declaration of Helsinki.

Patient and tissue samples

Tissue microarrays were purchased from a commercial chip company (Superchip, Shanghai, China). There were 93 patients, including 87 patients with esophageal carcinoma and normal esophageal tissue, and another 6 patients with only esophageal cancer tissue. There are a total of 180 points on the tissue microarray. The diameter of tissue pieces on the tissue microarray was 1.5 mm, and all points were overlaid with paraffin wax.

Among the 93 patients there were 77 men and 16 women. Ages ranged from 49 to 85 years; the average age was 65.8 years. All patients with pathological type are ESCC. The enrolled patients did not receive chemotherapy or radiotherapy before surgery. The patients underwent surgery between January 2009 and December 2010 year. The follow-up time extended to September 2014 (3.8-5.7 years). Overall survival was considered the time from radical surgery to death from any cause.

The tissue microarray contained clinicopathologic data (**Table 1**) that included: patient's age; gender; tumor size and location; tumor growth pattern; pathological type; pathological grade; tumor T, N, M and American Joint Committee on Cancer (AJCC) 7th edition staging; and postoperative total survival time. Seven patients had no T stage information. Five patients had no AJCC stage information.

Immunohistochemistry

Chips were baked in a hot oven incubator for 2 hours and then placed in xylene for 2 × 5 min incubations to deparaffinize the specimen. Chips were then transferred to 100%, 100%, 95%, 80%, and 70% successive ethanol washes every 5 min to rehydrate the specimen. Antigen retrieval was performed in a pressure cooker with citrate buffer (10 mM citrate and 0.05% Tween 20, pH 6.0). The chip was then incubated in 0.3% H_2O_2 in TBS for 15 min to suppress endogenous peroxidases.

The chip was incubated with an affinity-isolated polyclonal rabbit antibody against SIRT4 (HPA-029691, 1:100, Sigma, USA) at 4°C overnight. Secondary antibody was applied using a GTVision Kit (Gene Tech, Shanghai, China). The chip was stained with diaminobenzidine and then counterstained with hematoxylin. The chip was then dehydrated and sealed with coverslips in accordance with standard procedures. Tissues

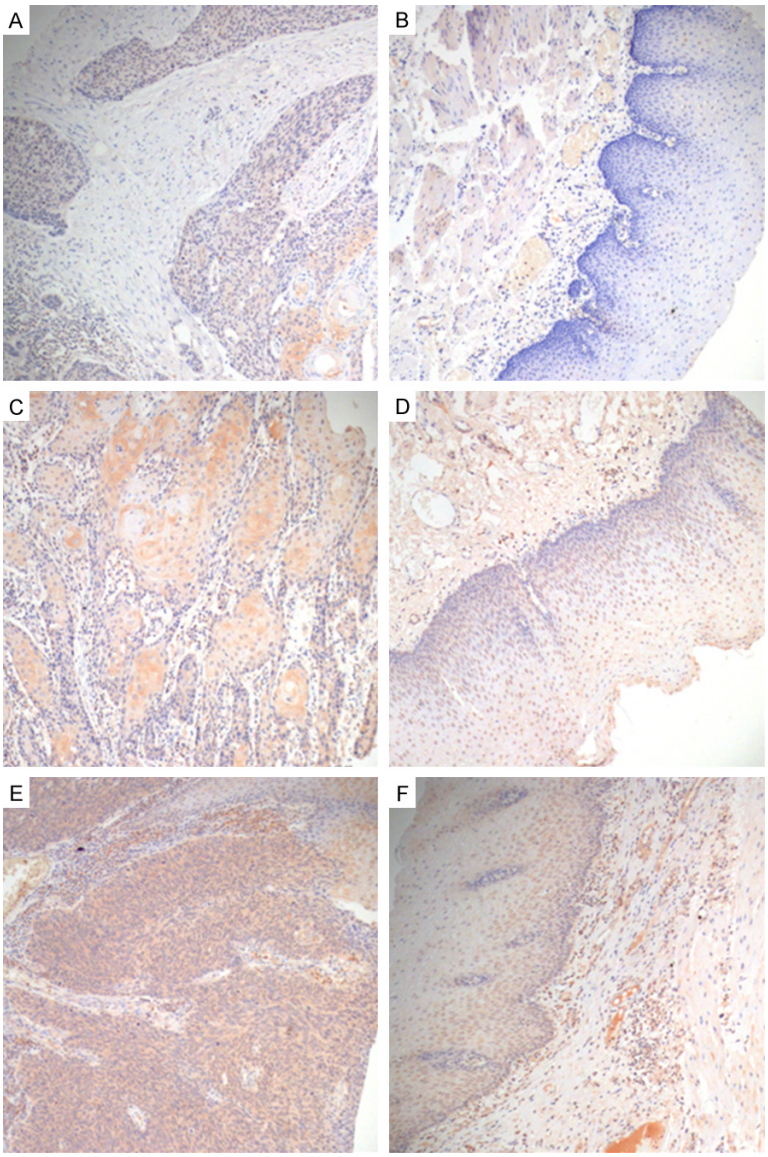

Figure 1. Representative immunohistochemical staining of SIRT4 protein in human esophageal carcinoma tissues. SIRT4 was expressed in the cytoplasm. The micrographs showed weak (A), medium (C) and strong (E) staining of SIRT4 in the esophageal carcinoma tissues. SIRT4 levels in corresponding adjacent normal tissues are shown in (B, D and F), respectively. Magnification 100 ×.

treated with the antibody vehicle were used as the negative control.

Two pathologists who were blinded to the patient information evaluated the SIRT4 immunostaining intensity under a light microscope. Each tissue point was assigned a score based on the staining intensity multiplied by the area of the stain [12]. The staining intensity was divided into 4 levels: 0, none; 1, weak; 2, moderate; or 3, strong. The assessment of staining area was based on the percentage of cells that were positively stained as follows: 0-0.5%; 1.5%-25%; 2.26%-50%; 3.51-75%; and 4, > 75%. The degree of staining was considered as: 0-5, low; or 6 to 12 points, high. The 2 pathologists rectified any disagreement in staining result evaluations through discussion.

Statistical analysis

Statistical analyses were performed using the statistical software SPSS 20.0 version. The paired t-test was used for the analysis of SIRT4 staining score differences between ESCC and normal tissues. The chi-squared test and Fisher's exact test were used to analyze the SIRT4 immunohistochemical staining results and the association between SIRT4 and the clinicopathological parameters. The Kaplan-Meier method (the log-rank test) was used for single-factor analysis. The Cox proportional hazards regression model was used to identify the independent prognostic factors. A *P*-value < 0.05 (2-tailed) was considered statistically significant.

Results

SIRT4 Expression in ESCC and adjacent normal tissues

SIRT4 was mainly expressed in the cell cytoplasm (**Figure 1**). The staining intensity of SIRT4 in ESCC tissues was stronger than that of adjacent normal tissues (median score: Cancer = 6.1 compared with Normal = 4.7, P < 0.001, **Figure 2A**).

Associations between SIRT4 levels and clinicopathological parameters

Associations between SIRT4 levels and clinicopathological features were evaluated using immunohistochemistry (**Table 1**). We found significant relationships between SIRT4 expres-

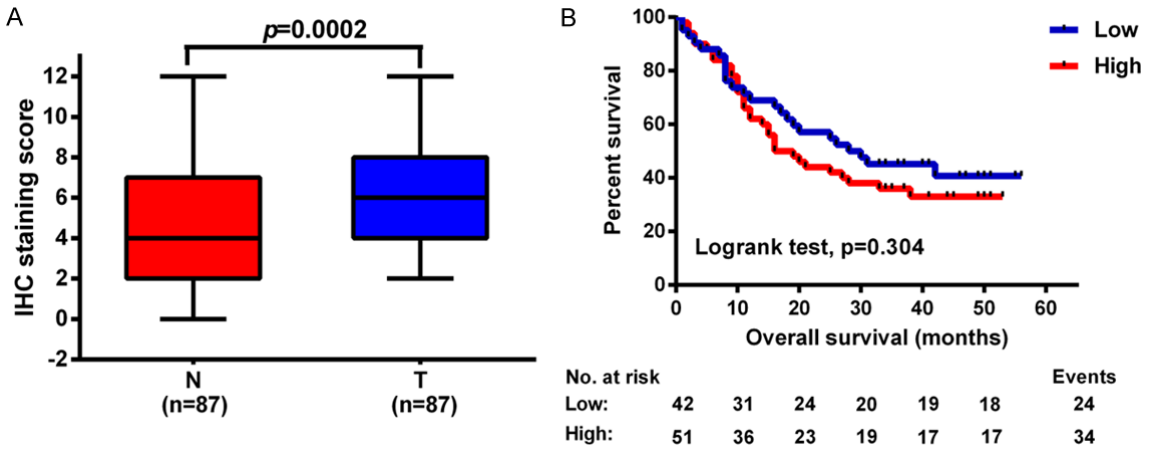

Figure 2. A. SIRT4 protein levels in 87 esophageal carcinoma tissues and paired adjacent normal esophageal tissues as measured by tissue microarray. SIRT4 protein levels were higher than in adjacent normal esophageal tissues. The boxes represent the interquartile range; whiskers represent the minimum-to-maximum range; bars represent the median. B. Kaplan-Meier curve comparing the patient survival times of those with low SIRT4 levels in esophageal cancer tissues and those with high levels; determined using tissue microarray. The total survival rate of patients with esophageal cancer with low SIRT4 levels was significantly lower than that of patients with high SIRT4 levels; P = 0.304, log-rank test.

sion and gender. Women with esophageal cancer, patients are more likely to appear SIRT4 high expression. However, SIRT4 levels were not associated with: age, tumor size, pathologic differentiation degree, tumor invasion depth (T), lymph node positive number (N), distant metastasis (M), or AJCC stage is statistically significant correlation (P > 0.05).

Association between SIRT4 levels and total survival time

The Kaplan-Meier analysis and log-rank test were used to investigate the prognostic value of SIRT4 levels on patient survival. In the univariate analysis, we found that depth of tumor invasion (T), lymph node metastasis (N) and AJCC stage were associated with overall survival (P = 0.030, < 0.016, and < 0.003, respectively, **Table 2**) in patients with ESCC. And we did not find a correlation between SIRT4 levels and the total survival time (P = 0.304). However, the average survival time of patients with high SIRT4 levels in esophageal cancer tissues was lower than that of patients with low levels (mean survival time: SIRT4 High = 26.2 months compared with SIRT4 Low = 32.1 months) (**Figure 2B**).

COX regression analysis was used to analyze the independent prognostic factors for overall survival time of patients with ESCC. After adjusting the prognostic factors in univariate analysis, there was a significant correlation between AJCC stage and total survival time (data not show).

Discussion

In the present study, we analyzed the levels of SIRT4 in ESCC tissues and the association between them and the clinicopathological parameters of patients with ESCC. Our results showed that SIRT4 was mainly located in the cell cytoplasm, and levels were higher than that of normal matched tissues. In addition to women with esophageal cancer, patients are more likely to appear SIRT4 high expression, SIRT4 expression intensity and the rest of the esophageal cancer clinicopathological parameters are not related. The average survival time of patients with high SIRT4 levels in esophageal cancer tissues was lower than that of patients with low levels.

Based on the results of the present study and others, the different SIRT family members have roles that depend on the specific tissue and tumor type [13]. For example, the presence of SIRT1 in gastric [14], colon [15], prostate [16], skin [17] and other tumors suggests that it has a role in promoting tumor development in these cancers. However, other studies have found that SIRT1 levels are lower in breast cancer

Table 2. Univariate analysis of SIRT4 expression and clinicopathologic variables in 93 patients with ESCC

			Overall survival, mo		
		All cases, n	Mean	Median	*P**
Age, y	≤ 60	27	30.0	27.0	0.834
	> 60	66	28.1	20.0	
Gender	Male	76	28.1	20.0	0.190
	Female	16	34.1	NR	
Tumor size, cm	≤ 5	61	28.7	20.0	0.726
	> 5	32	29.2	28.0	
Differentiation	Well	14	34.7	21.0	0.526
	Moderate	60	28.1	19.0	
	Poor	19	27.4	17.0	
T stage	T1-T2	22	38.1	NR	**0.030**
	T3-T4	64	25.7	17.0	
N stage	N0	45	35.2	42.0	**0.016**
	N1-N3	48	23.2	15.0	
M stage	M0	93	38.8	28.0	0.408
	M1	0	28.2	29.0	
AJCC stage	I	39.4	49.3	NR	**0.005**
	II	34.0	27.4	31.0	
	III	20.0	20.2	14.0	
SIRT4 levels	Low	32	32.1	28.0	0.304
	High	61	26.2	16.0	

Bold values are statistically significant (P < 0.05). NR, not reached. *log-rank test.

than in normal tissues [18], and in an APCmin/+ mouse model of colon cancer can inhibit the formation of intestinal tumors [19]. This is similar to SIRT2, which was shown to be downregulated in breast cancer [20], glioma [21], and skin cancer [22], while SIRT2 levels were elevated in acute myeloid leukemia [23] and prostate cancer [24]. Thus, we cannot easily extrapolate conclusions from the observations of one tumor type to others.

There have been a limited number of studies regarding the SIRT4 gene in oncology, but all agree that it functions as a tumor suppressor. For example, Jeong [6] concluded that SIRT4 suppresses tumor formation by inhibiting glutamine metabolism. Furthermore, overexpression of SIRT4 could inhibit the growth of HeLa cells, SIRT4 knockout mouse embryo fibroblasts formed larger tumors in nude mice, and SIRT4 knockout mice spontaneously generated lung cancer, liver cancer, breast cancer, and lymphoma. Csibi [7] also found that overexpression of SIRT4 could inhibit the growth of human colon cancer cells (DLD-1) and human prostate cancer cells (DU145). Jeong [25] found that SIRT4 inhibited the growth of Myc-induced B cell lymphoma.

Our previous study found that SIRT4 levels were downregulated in gastric cancer and colon cancer tissues and were associated with pathological grading and other clinicopathological parameters [8, 10]. In the present experiment, however, we found that SIRT4 protein levels were higher in esophageal cancer tissues than adjacent normal esophageal tissues. Moreover, the average survival time of patients with high SIRT4 levels in esophageal cancer tissues was lower than that of patients with low levels. This would suggest that the SIRT4 gene in esophageal cancer is an oncogene.

Our present results are somewhat contradictory to recent literature. Nakahara [11] found that low SIRT4 levels were associated with poorer prognosis in esophageal cancer, and they also found that reduction in SIRT4 could promote esophageal cancer cell proliferation and migration. But they used paraffin sections, and the accuracy of immunohistochemistry is not equal to that of a tissue microarray; different races, samples, and different antibodies will also result in different results.

Current research on SIRT4 supports that it can inhibit tumor metabolism, especially the inhibition of glutamine metabolism [6, 7, 26], and acts as a tumor suppressor gene. It is thought that SIRT4 is a gate-keeper of cellular energy metabolism [26]. Indeed, altered energy metabolism is a feature of tumors [27]. Tumor cells and normal cells have distinct metabolic patterns. Tumor cells often appear to enhance glucose and glutamine metabolism to provide the energy for growth of the tumor [28, 29]. Because the higher the degree of malignancy, the faster the proliferation, the corresponding energy demand is also stronger. Thus, the ability of SIRT4 to inhibit glutamine metabolism is in accord with its identification as a tumor sup-

pressor gene. However, there has been little research on the mechanism of SIRT4. Like other members of the SIRT family, SIRT4 may be a component of a complex regulatory network, and it may act variously as a tumor suppressor gene or oncogene. Therefore, the SIRT4 regulatory network in tumors warrants further research, to facilitate in-depth understanding of its role in cancer.

In the present study, we found that the total survival time of esophageal cancer patients with high SIRT4 levels was shorter than that of patients with low levels, but the difference was not statistically significant. This may be due to the small size of the sample population.

In summary, our results indicate SIRT4 may participant in the development of ESCC, and SIRT4 protein in tumors may have a complex mechanism of action.

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Disclosure of conflict of interest

None.

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References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Murugaesu N, Wilson GA, Birkbak NJ, Watkins TB, McGranahan N, Kumar S, Abbassi-Ghadi N, Salm M, Mitter R, Horswell S, Rowan A, Phillimore B, Biggs J, Begum S, Matthews N, Hochhauser D, Hanna GB and Swanton C. Tracking the genomic evolution of esophageal adenocarcinoma through neoadjuvant chemotherapy. Cancer Discov 2015; 5: 821-831.

[3] Secrier M and Fitzgerald RC. Signatures of Mutational Processes and Associated Risk Factors in Esophageal Squamous Cell Carcinoma: a Geographically Independent Stratification Strategy? Gastroenterology 2016; 150: 1080-3.

[4] Finkel T, Deng CX and Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. Nature 2009; 460: 587-591.

[5] Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW and Guarente L. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. Cell 2006; 126: 941-954.

[6] Jeong SM, Xiao C, Finley LW, Lahusen T, Souza AL, Pierce K, Li YH, Wang X, Laurent G, German NJ, Xu X, Li C, Wang RH, Lee J, Csibi A, Cerione R, Blenis J, Clish CB, Kimmelman A, Deng CX and Haigis MC. SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. Cancer Cell 2013; 23: 450-463.

[7] Csibi A, Fendt SM, Li C, Poulogiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM, Parkhitko A, Morrison T, Henske EP, Haigis MC, Cantley LC, Stephanopoulos G, Yu J and Blenis J. The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. Cell 2013; 153: 840-854.

[8] Huang G, Cui F, Yu F, Lu H, Zhang M, Tang H and Peng Z. Sirtuin-4 (SIRT4) is downregulated and associated with some clinicopathological features in gastric adenocarcinoma. Biomed Pharmacother 2015; 72: 135-139.

[9] Miyo M, Yamamoto H, Konno M, Colvin H, Nishida N, Koseki J, Kawamoto K, Ogawa H, Hamabe A, Uemura M, Nishimura J, Hata T, Takemasa I, Mizushima T, Doki Y, Mori M and Ishii H. Tumour-suppressive function of SIRT4 in human colorectal cancer. Br J Cancer 2015; 113: 492-499.

[10] Huang G, Cheng J, Yu F, Liu X, Yuan C, Liu C, Chen X and Peng Z. Clinical and therapeutic significance of sirtuin-4 expression in colorectal cancer. Oncol Rep 2016; 35: 2801-10.

[11] Nakahara Y, Yamasaki M, Sawada G, Miyazaki Y, Makino T, Takahashi T, Kurokawa Y, Nakajima K, Takiguchi S, Mimori K, Mori M and Doki Y. Downregulation of SIRT4 Expression Is Associated with Poor Prognosis in Esophageal Squamous Cell Carcinoma. Oncology 2016; 90: 347-55.

[12] Chen J, Zhang B, Wong N, Lo AW, To KF, Chan AW, Ng MH, Ho CY, Cheng SH, Lai PB, Yu J, Ng HK, Ling MT, Huang AL, Cai XF and Ko BC. Sirtuin 1 is upregulated in a subset of hepatocellular carcinomas where it is essential for telomere maintenance and tumor cell growth. Cancer Res 2011; 71: 4138-4149.

[13] Roth M and Chen WY. Sorting out functions of sirtuins in cancer. Oncogene 2014; 33: 1609-20.

[14] Cha EJ, Noh SJ, Kwon KS, Kim CY, Park BH, Park HS, Lee H, Chung MJ, Kang MJ, Lee DG, Moon WS and Jang KY. Expression of DBC1 and SIRT1 is associated with poor prognosis of gastric carcinoma. Clin Cancer Res 2009; 15: 4453-4459.

[15] Stunkel W, Peh BK, Tan YC, Nayagam VM, Wang X, Salto-Tellez M, Ni B, Entzeroth M and Wood J. Function of the SIRT1 protein deacetylase in cancer. Biotechnol J 2007; 2: 1360-1368.

[16] Huffman DM, Grizzle WE, Bamman MM, Kim JS, Eltoum IA, Elgavish A and Nagy TR. SIRT1 is significantly elevated in mouse and human prostate cancer. Cancer Res 2007; 67: 6612-6618.

[17] Hida Y, Kubo Y, Murao K and Arase S. Strong expression of a longevity-related protein, SIRT1, in Bowen's disease. Arch Dermatol Res 2007; 299: 103-106.

[18] Wang RH, Sengupta K, Li C, Kim HS, Cao L, Xiao C, Kim S, Xu X, Zheng Y, Chilton B, Jia R, Zheng ZM, Appella E, Wang XW, Ried T and Deng CX. Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cell 2008; 14: 312-323.

[19] Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, Campbell J, Bhimavarapu A, Luikenhuis S, de Cabo R, Fuchs C, Hahn WC, Guarente LP and Sinclair DA. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS One 2008; 3: e2020.

[20] Kim HS, Vassilopoulos A, Wang RH, Lahusen T, Xiao Z, Xu X, Li C, Veenstra TD, Li B, Yu H, Ji J, Wang XW, Park SH, Cha YI, Gius D and Deng CX. SIRT2 maintains genome integrity and suppresses tumorigenesis through regulating APC/C activity. Cancer Cell 2011; 20: 487-499.

[21] Hiratsuka M, Inoue T, Toda T, Kimura N, Shirayoshi Y, Kamitani H, Watanabe T, Ohama E, Tahimic CG, Kurimasa A and Oshimura M. Proteomics-based identification of differentially expressed genes in human gliomas: down-regulation of SIRT2 gene. Biochem Biophys Res Commun 2003; 309: 558-566.

[22] Ming M, Qiang L, Zhao B and He YY. Mammalian SIRT2 inhibits keratin 19 expression and is a tumor suppressor in skin. Exp Dermatol 2014; 23: 207-209.

[23] Dan L, Klimenkova O, Klimiankou M, Klusman JH, van den Heuvel-Eibrink MM, Reinhardt D, Welte K and Skokowa J. The role of sirtuin 2 activation by nicotinamide phosphoribosyltransferase in the aberrant proliferation and survival of myeloid leukemia cells. Haematologica 2012; 97: 551-559.

[24] Hou H, Chen W, Zhao L, Zuo Q, Zhang G, Zhang X, Wang H, Gong H, Li X, Wang M, Wang Y and Li X. Cortactin is associated with tumour progression and poor prognosis in prostate cancer and SIRT2 other than HADC6 may work as facilitator in situ. J Clin Pathol 2012; 65: 1088-1096.

[25] Jeong SM, Lee A, Lee J and Haigis MC. SIRT4 suppresses tumor formation in genetic models of Myc-induced B cell lymphoma. J Biol Chem 2014; 289: 4135-44.

[26] Mathias RA, Greco TM, Oberstein A, Budayeva HG, Chakrabarti R, Rowland EA, Kang Y, Shenk T and Cristea IM. Sirtuin 4 is a lipoamidase regulating pyruvate dehydrogenase complex activity. Cell 2014; 159: 1615-1625.

[27] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

[28] Daye D and Wellen KE. Metabolic reprogramming in cancer: unraveling the role of glutamine in tumorigenesis. Semin Cell Dev Biol 2012; 23: 362-369.

[29] Tennant DA, Duran RV and Gottlieb E. Targeting metabolic transformation for cancer therapy. Nat Rev Cancer 2010; 10: 267-277.