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SIRT3 acts as a novel biomarker for the diagnosis of lung cancer

A retrospective study

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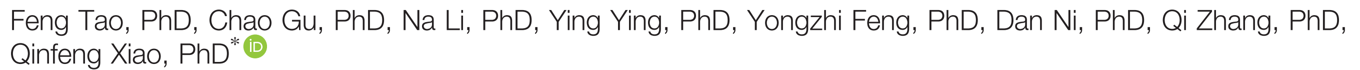

Abstract

Lung cancer (LC) is a prevalent malignancy worldwide with increased morbidity and mortality. Mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase sirtuin-3 (SIRT3) has been reported to be involved in tumorigenesis. In this retrospective study, we measured the expression and diagnostic value of SIRT3 in LC patients.

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to measure serum *SIRT3* mRNA level in 150 LC patients and 52 healthy volunteers. SIRT3 protein level was detected using western blot for 84 pairs of LC and adjacent normal tissues. The association of *SIRT3* mRNA level with clinical parameters of LC patients was estimated via chi-square test. Receiver operating characteristic curve (ROC) was plotted to evaluate the diagnostic performance of serum *SIRT3* in LC patients.

SIRT3 mRNA and protein levels were significantly decreased in LC tissues and serum samples, compared with corresponding controls ($P < .05$). Moreover, the expression of *SIRT3* mRNA was negatively associated with tumor size ($P = .002$), tumor node metastasis stage ($P < .001$), and metastasis ($P < .001$). ROC curve demonstrated that serum *SIRT3* could distinguish LC patients from healthy individuals, with an area under the curve of 0.918. The optimal cutoff value was 3.12, reaching a sensitivity of 86.4%, and a specificity of 94%.

SIRT3 expression is significantly down-regulated in LC serum and tissues. *SIRT3* may be employed as a promising biomarker in the early diagnosis of LC.

Abbreviations: AUC = area under the curve, LC = lung cancer, qRT-PCR = quantitative real-time polymerase chain reaction, ROC = receiver operating characteristic, Sir2 = silent information regulator 2, SIRT3 = sirtuin-3, TNM = tumor node metastasis.

Keywords: diagnosis, lung cancer, *SIRT3*

1. Introduction

Lung cancer (LC) imposes a great threat to human health because of its high morbidity and mortality.[1] According to incomplete statistics, LC-related deaths account for nearly 29% of total cancer-related deaths annually.[2–4] The 5-year survival rate of LC patients is less than 15%.[5,6] Early diagnosis is key for the survival of LC patients. Unfortunately, symptoms in early LC are mild, even without any discomfort. Most of the patients are diagnosed at advanced stages, missing the optimal opportunity for surgical treatment.[6,7] Thus, it is urgent to explore effective diagnostic biomarkers for LC.

Mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase sirtuin-3 (*SIRT3*)[8] is a well-known tumor suppressor and participates in multiple metabolic processes through deacetylating downstream protein substrates.[9] The dysregulation of *SIRT3* has been reported in multiple diseases, such as cancers, cardiovascular diseases, and diabetes.[10]*SIRT3* is a member of the sirtuin family (SIRT1-7) whose members function as metabolic sensors to connect their enzyme activities with cell energy and redox state through NAD^+ cofactor.[11,12] Sirtuin family plays important roles in a variety of cellular processes, such as oxidative stress, genomic stability, apoptosis, and protein and fatty acid metabolism.[13,14] Human *SIRT3* gene locates on chromosome 11p15.5, and encodes a 44KD-protein.[15,16]*SIRT3* has been reported to exert inhibitory effects against many diseases, but its diagnostic value in LC is rarely reported.

In the present study, we aimed to detect the expression and diagnostic value of serum *SIRT3* in LC patients.

2. Methods and materials

2.1. Study design and setting

The present study was a retrospective investigation. A total of 150 LC patients and 52 healthy individuals were enrolled in the study from May, 2015 to May, 2017. Blood samples were

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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collected from all participants, while LC and adjacent normal tissues were randomly obtained from 84 LC patients.

2.2. Participants

The patients in the current study must meet the following criteria: 1) with an age of ≥18 years; 2) pathologically diagnosed as primary LC without other malignancies; 3) experiencing no anti-tumor treatments, either chemotherapy, surgery, radiotherapy, or targeted treatments; and 4) with available blood specimens and clinical records. With any one of the following conditions, cases would be excluded: 1) suffering recurrent LC; 2) having benign lung diseases; and 3) with severe heart, liver and/or kidney diseases, inflammation diseases, history of asthma, and recent respiratory infections.

According to the tumor node metastasis (TNM) cancer staging system (2009) set by the Union of International Cancer Control, tumors were classified. Of all LC patients, 84 experienced surgery operation and their diseased tissues and adjacent normal tissues (4 cm away from cancer lesions) were obtained. In addition, 52 healthy volunteers were recruited as healthy controls. The healthy controls did not show any abnormalities in physical examinations and had no malignancy history or lung diseases. Moreover, the healthy controls were matched with LC patients in age and gender. Blood specimens (5 mL venous blood) were collected from LC patients on the day of diagnosis before accepting any surgery or therapy. Basic data of the patients were recorded, including age, sex, tumor size, diseased region, pathologic types, TNM stage, and metastasis status.

2.3. Serum preparation and the extraction of RNA

Serum specimen was separated from whole blood via centrifugation. Neither clinical nor pathological data were disclosed during detection. Total RNAs were isolated from serum and tissue samples using TRIzol (Life Technologies, Carlsbad, CA, USA). Only RNA samples with an OD A260/A280 ratio close to 2.0 were used in subsequent analysis.

2.4. Quantitative real-time polymerase chain reaction

This process proceeded in a blinded way. Reverse transcription was conducted with 100 ng RNA using PrimeScript RT (Takara, Dalian, China), according to the manufacturer's instructions. Then, quantitative real-time polymerase chain reaction (qRT-PCR) reaction was completed adopting Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *GAPDH* was employed as the internal reference. The relative expression of *SIRT3* was normalized to *GAPDH* and calculated using $2^{-\Delta\Delta Ct}$ method. Each sample was checked in triplicate.

2.5. Western blot

Total proteins were extracted from tissue samples using the One Step Animal Tissue Active Protein Extraction Kit (Sangon, Shanghai). Then, the proteins were lysed and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto PVC membrane. Next, the membranes were blocked, applying TBST with 5% nonfat milk at 4 °C for 1 night. The primary antibody anti-SIRT3 was diluted with block solution to proper concentration and then incubated at 4 °C overnight. Then, a secondary antibody was added and incubated for 2 h at 4 °C. ECL Western blotting kit (Millipore, Boston, MA, USA) was used to visualize the target band of *SIRT3*. Each test had 3 repeats.

2.6. Ethics approval

This study was approved by the Ethics Committee of The First Affiliated Hospital of Jiaxing University (Ethical reference number: 2017KY0402) in October, 2017. All the patients or their families signed written informed consent before sample collection.

2.7. Statistical analysis

All statistical analyses were performed using Origin Pro 9.0 software. Statistical power was calculated using G*Power software, and statistical power was fine when obtained values ≥0.8. Continuous data were summarized as mean ± SD. Differences in *SIRT3* mRNA and protein levels between the 2 groups were analyzed using Student *t* test. Chi-square test was adopted to estimate the relationship between serum *SIRT3* expression and clinical characteristics of LC cases. To determine the diagnostic value of serum *SIRT3* in LC, the receiver operating characteristic (ROC) curve was plotted, and relevant results were estimated using the area under the curve (AUC) combining with sensitivity and specificity. P values $<.05$ indicated the statistical significance of the results.

3. Results

3.1. The expression level of SIRT3

In our study, serum samples were collected from 150 LC patients and 52 healthy controls. The paired LC and adjacent normal tissues were collected from 84 LC patients receiving surgical operations. qRT-PCR was used to estimate *SIRT3* mRNA expression in serum and tissue specimens. Meanwhile, the protein expression of SIRT3 was detected via western blot. As shown in Figure 1A, serum level of *SIRT3* was significantly lower in LC patients than in healthy controls ($P<.01$). The expression level of *SIRT3* was significantly higher in LC patients at stage I–II than in stage III–IV LC patients ($P<.01$, Fig. 1B). In metastatic patients, *SIRT3* expression level was significantly decreased, compared with non-metastatic patients ($P<.01$, Fig. 1C). Similarly, the expression level of *SIRT3* mRNA was also obviously lower in LC tissues than in adjacent normal tissues (2.054 ± 0.937 vs 4.077 ± 0.952, $P<.05$, Fig. 1D). Western blot analysis demonstrated that the expression of SIRT3 protein was weak in LC tissues, compared with adjacent normal tissues (Fig. 1E). Based on the expression patterns of *SIRT3* mRNA in LC and healthy groups, the statistical power of study subjects was 1.00, revealing high credibility of our analysis.

3.2. Relationship between SIRT3 and clinicopathological characteristics in LC

Clinicopathological characteristics of LC patients were shown in Table 1. Chi-square test showed that *SIRT3* mRNA level was negatively associated with tumor size ($P=.001$), TNM stage ($P<.001$), and metastasis in LC patients ($P<.001$). However, there was no significant association between *SIRT3* expression

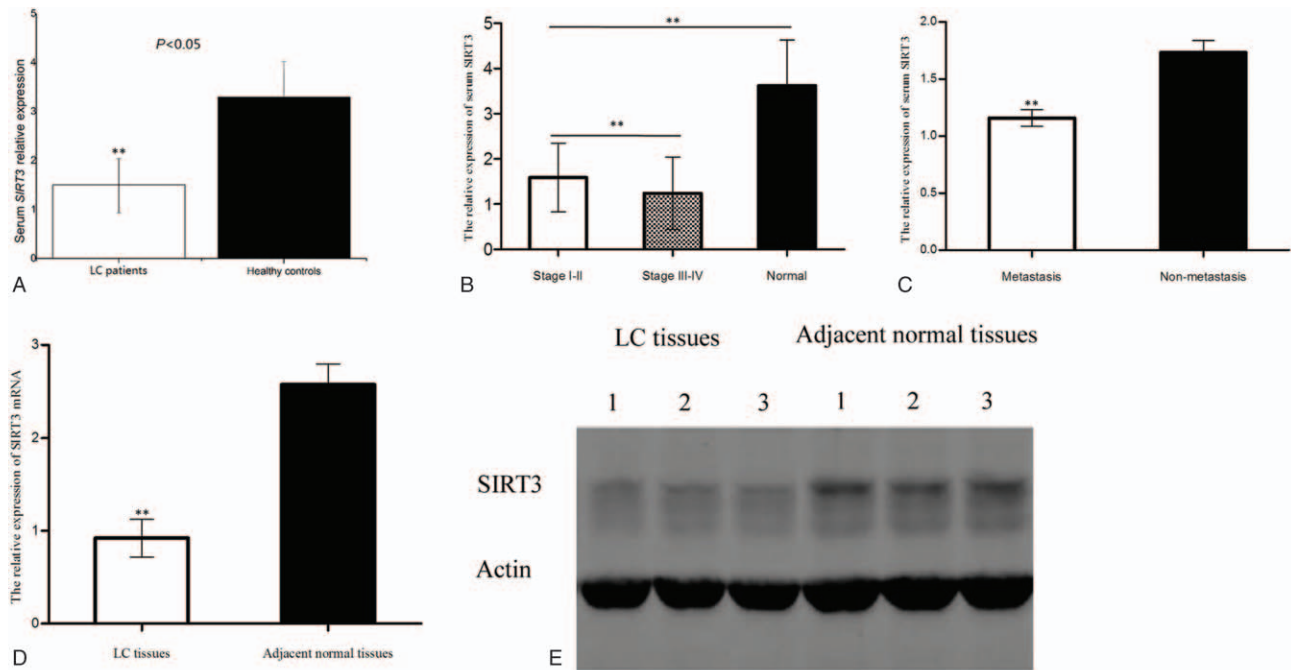

Figure 1. The expression patterns of *SIRT3* in LC. (A) Serum *SIRT3* expression level was decreased in LC patients and healthy controls, adopting qRT-PCR methods; (B) serum *SIRT3* expression level was significantly lower in cases at stage III–IV than in those at stage I–II; (C) serum *SIRT3* expression level was also significantly lower in metastatic patients than in non-metastatic ones; (D) and (E) *SIRT3* mRNA and protein expression levels were obviously decreased in LC tissues, compared with adjacent normal tissues. $^{**}P<.01$ meant the significant difference between compared 2 sides. LC=lung cancer, qRT-PCR=quantitative real-time polymerase chain reaction, SIRT3=Sirtuin-3.

Table 1

The expression of *SIRT3* and clinicopathological features in LC patients.

Features	No. n=150	*SIRT3* expression Low (n=84)	High (n=66)	*P* values
Age (years)				
<60	79	47	32	.363
≧60	71	37	34	
Gender				
Male	94	50	44	.369
Female	56	34	22	
Tumor size				
<3cm	61	25	36	.002
≧3cm	89	59	30	
Diseased region				
Central type	76	40	36	.400
Peripheral	74	44	30	
Pathologic types				
Squamous carcinoma	43	20	23	.123
Adenocarcinoma	66	43	23	
Small cell lung cancer	41	21	20	
TNM stage				
I–II	64	25	39	<.001
III–IV	86	59	27	
Metastasis				
Yes	91	65	26	<.001
No	59	19	40	

LC=lung cancer, SIRT3=Sirtuin-3, TNM=tumor node metastasis.

level and age, gender, diseased region, or pathologic types (all $P>.05$; Table 1).

3.3. Diagnostic value of SIRT3 in LC

ROC curve showed that *SIRT3* expression could distinguish LC patients from healthy controls with a sensitivity and specificity of 86.4% and 94%, respectively. Meanwhile, the AUC was 0.918 (Figure 2). The cutoff value for serum *SIRT3* mRNA diagnosing LC was 3.12.

4. Discussion

Early diagnosis and timely treatment are very important for the prognosis of LC.[17,18] Currently, the diagnosis of LC mainly depends on histology and iconography, resulting in its delay.[19] Sensitive and efficient biomarkers are in urgent need to facilitate the early diagnosis of LC. In the current study, we estimated the diagnostic significance of serum *SIRT3* in LC. The results suggested that the down-regulation of *SIRT3* showed a negative association with tumor size, TNM stage, and metastasis. Serum *SIRT3* level might be a potential biomarker for the non-invasive detection of LC.

SIRT3, a member of the sirtuin family, plays a critical role in cancers.[20,21] Accumulating evidence have demonstrated that *SIRT3* plays a regulatory role in mitochondria, including metabolism, ATP production, responses to oxidative stress, and apoptosis.[22–24] Previous studies have reported that the

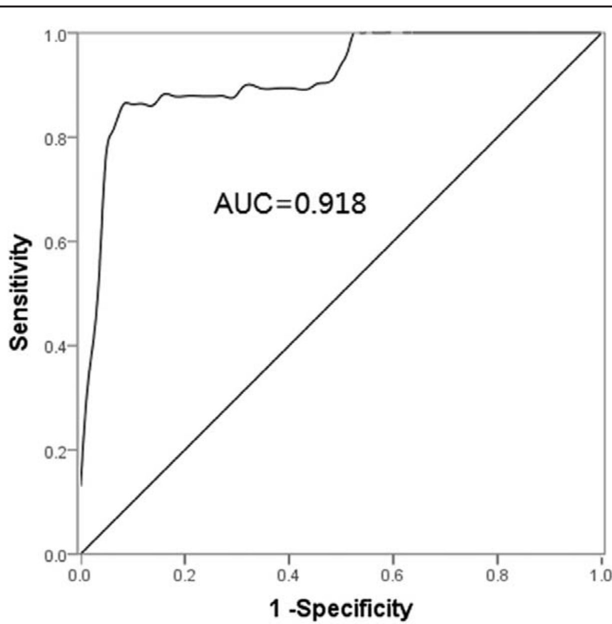

Figure 2. ROC analysis for the accuracy of serum *SIRT3* in distinguishing LC patients from healthy volunteers. AUC = area under the curve, SIRT3 = Sirtuin-3, ROC = receiver operating characteristic.

expression of *SIRT3* was decreased in human lung adenocarcinoma tissues, and that *SIRT3* exhibited an anti-tumor effect on lung adenocarcinoma cells.[25] However, the potential role of *SIRT3* in LC diagnosis is rarely known.

In our study, we found that the expression level of *SIRT3* was significantly lower in LC patients than in healthy volunteers. The results were in line with previous studies. For example, Zhang et al claimed that the expression of *SIRT3* was significantly lower in hepatocellular carcinoma tissues than in normal liver tissues and cirrhotic tissues.[26] Finley et al found *SIRT3* expression was reduced in human breast cancer and its overexpression repressed glycolysis and proliferation in breast cancer cells.[27] In the study of Xiao et al, the expression of *SIRT3* showed a down-regulated trend in lung adenocarcinoma cells compared with normal cells.[25] But some contrary results were also reported. The study performed by Xiong et al showed that *SIRT3* protein and mRNA levels were significantly increased in non-small cell lung cancer.[28] Yang et al found that the expression of *SIRT3* in non-small cell lung cancer tissues was obviously higher than that in normal tissues.[29] The controversy may be attributed to different pathological types. Across pathological types of cancers, *SIRT3* might trigger different key proteins and signaling pathways, thus initiating oncogenic or anti-tumor actions.[28] In addition, different causes for cancers might also contribute to the discrepancy. Reportedly, *SIRT3* might act as an oncogene through enhancing reactive oxygen species resistance of cancer cells. However, under stress conditions, *SIRT3* could induce cell apoptosis, thus achieving anti-tumor action.[30] *SIRT3* plays dual roles in tumorigenesis.

In addition, we evaluated the relationship between *SIRT3* expression and clinicopathological features of LC patients. The results showed that *SIRT3* mRNA expression was significantly correlated with tumor size, TNM stage, and metastasis in LC patients, which suggested that *SIRT3* might be involved in LC progression. Moreover, we also investigated the diagnostic value of *SIRT3* in LC. ROC curve demonstrated that the expression of *SIRT3* could distinguish LC patients from healthy individuals. *SIRT3* might be an indicator in the early diagnosis of LC. The present research showed the detailed expression levels of *SIRT3* mRNA in both tissues and serum for patients at different grades, as well as the protein level in tissues. Besides, the ROC curve presented a high diagnostic value of *SIRT3* in LC patients.

However, several shortcomings in the current study should be stated. Firstly, the retrospective design might cause bias in sample collection. Secondly, all individuals in the control group were healthy persons, and the expression of *SIRT3* in cases with benign lung diseases or other malignancies remained unclear. Whether the expression of *SIRT3* was specific to LC was not determined. In other words, whether serum *SIRT3* could distinguish LC patients from other cancer cases or benign lung disease sufferers was not decided. Thirdly, the protein level of SIRT3 was only detected adopting western blot analysis, and immunohistochemistry was necessary to verify obtained results. Besides, due to lacking follow-up information, the clinical value of *SIRT3* for prognosis evaluation among LC patients was not explored. Therefore, further investigations are required to verify and improve our findings.

In conclusion, *SIRT3* acts as a tumor suppressor in LC and maybe an auxiliary biomarker in early diagnosis of LC.

Author contributions

Conceptualization: Feng Tao, Chao Gu.
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Funding acquisition: Ying Ying, Qi Zhang.
Investigation: Na Li.
Project administration: Na Li.
Resources: Na Li, Dan Ni.
Software: Ying Ying, Dan Ni.
Validation: Ying Ying.
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Writing – review & editing: Chao Gu, Na Li, Qi Zhang.

References

[1] Parente Lamelas I, Abal Arca J, Firvida Perez JL. Directed therapies in lung cancer: new hope? Arch Bronconeumol 2012;48:367–71.
[2] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
[3] Bunn PA Jr. Worldwide overview of the current status of lung cancer diagnosis and treatment. Arch Pathol Lab Med 2012;136:1478–81.
[4] Sanchez de Cos Escuin J, Disdier Vicente C, Corral Penafiel J, Riesco Miranda JA, Sojo González MA, Masa Jiménez JF. [Overall long-term survival in lung cancer analyzed in 610 unselected patients]. Arch Bronconeumol 2004;40:268–74.
[5] Hueto Perez De Heredia J, Cebollero Rivas P, Cascante Rodrigo JA, et al. Evaluation of the use of a rapid diagnostic consultation of lung cancer. Delay time of diagnosis and therapy. Arch Bronconeumol 2012;48: 267–73.
[6] Escuin JS. [Lung cancer in Spain. Current epidemiology, survival, and treatment]. Arch Bronconeumol 2009;45:341–8.
[7] Santos-Martinez MJ, Curull V, Blanco ML, et al. [Lung cancer at a university hospital: epidemiological and histological characteristics of a recent and a historical series]. Arch Bronconeumol 2005;41:307–12.
[8] Huang KH, Hsu CC, Fang WL, et al. SIRT3 expression as a biomarker for better prognosis in gastric cancer. World J Surg 2014;38:910–7.
[9] Desouki MM, Doubinskaia I, Gius D, Abdulkadir SA. Decreased mitochondrial SIRT3 expression is a potential molecular biomarker associated with poor outcome in breast cancer. Human Pathol 2014;45: 1071–7.

[10] Yan SM, Han X, Han PJ, Chen HM, Huang LY, Li Y. SIRT3 is a novel prognostic biomarker for esophageal squamous cell carcinoma. Med Oncol 2014;31:103.
[11] Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000;403:795–800.
[12] Guarente L. Sir2 links chromatin silencing, metabolism, and aging. Genes Dev 2000;14:1021–6.
[13] Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. Oncogene 2007;26:5489–504.
[14] Haigis MC, Guarente LP. Mammalian sirtuins – emerging roles in physiology, aging, and calorie restriction. Genes Dev 2006;20:2913–21.
[15] Alhazzazi TY, Kamarajan P, Verdin E, Kapila YL. SIRT3 and cancer: tumor promoter or suppressor? Biochim Biophys Acta 2011;1816:80–8.
[16] Schwer B, North BJ, Frye RA, Ott M, Verdin E. The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. J Cell Biol 2002;158:647–57.
[17] Nair A, Klusmann MJ, Jogeesvaran KH, Grubnic S, Green SJ, Vlahos I. Revisions to the TNM staging of non-small cell lung cancer: rationale, clinicoradiologic implications, and persistent limitations. Radiographics 2011;31:215–38.
[18] Nwogu CE, Groman A, Fahey D, et al. Number of lymph nodes and metastatic lymph node ratio are associated with survival in lung cancer. Ann Thorac Surg 2012;93:1614–9. discussion 1619-1620.
[19] Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
[20] Schumacker PT. SIRT3 controls cancer metabolic reprogramming by regulating ROS and HIF. Cancer Cell 2011;19:299–300.
[21] Kim HS, Patel K, Muldoon-Jacobs K, et al. SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer Cell 2010;17:41–52.
[22] Yang M, Yang C, Pei Y. Effects of downregulation of SIRT3 expression on proliferation and apoptosis in esophageal squamous cell carcinoma EC9706 cells and its molecular mechanisms. Biomed Mater Eng 2014;24:3883–90.
[23] Ahn BH, Kim HS, Song S, et al. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc Natl Acad Sci U S A 2008;105:14447–52.
[24] Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A, Gupta MP. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. J Clin Invest 2009;119:2758–71.
[25] Xiao K, Jiang J, Wang W, et al. Sirt3 is a tumor suppressor in lung adenocarcinoma cells. Oncol Rep 2013;30:1323–8.
[26] Zhang B, Qin L, Zhou CJ, Liu YL, Qian HX, He SB. SIRT3 expression in hepatocellular carcinoma and its impact on proliferation and invasion of hepatoma cells. Asian Pac J Trop Med 2013;6:649–52.
[27] Finley LW, Carracedo A, Lee J, et al. SIRT3 opposes reprogramming of cancer cell metabolism through HIF1alpha destabilization. Cancer Cell 2011;19:416–28.
[28] Xiong Y, Wang M, Zhao J, et al. SIRT3 is correlated with the malignancy of non-small cell lung cancer. Int J Oncol 2017;50:903–10.
[29] Yang GC, Fu BC, Zhang DY, et al. The expression and related clinical significance of SIRT3 in non-small-cell lung cancer. Dis Markers 2017;2017:8241953.
[30] Torrens-Mas M, Oliver J, Roca P, Sastre-Serra J. SIRT3: oncogene and tumor suppressor in Cancer. Cancers 2017;9:90.