Clinical Report

Downregulation of SIRT6 is associated with poor prognosis in patients with non-small cell lung cancer

Bojin Zhu1, Yongjin Yan2, Boyun Shao1, Luwen Tian1 and Weihua Zhou1

Abstract

Objective: To explore the prognostic significance of nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase sirtuin-6 (SIRT6), encoded by the sirtuin 6 (SIRT6) gene, in a population of Chinese Han patients with non-small cell lung cancer (NSCLC).

Methods: Cancer tissues and normal lung tissues (>5 cm adjacent to cancer tissue) were collected from Chinese Han patients with NSCLC. Expression levels of SIRT6 and histone H3-acetyl K56 (H3K56), in cancer and normal lung tissues from patients with NSCLC, were detected by reverse-transcription polymerase chain reaction, Western blot and immunohistochemistry. Correlations between SIRT6 expression and various clinicopathologic features were investigated.

Results: Out of 86 patients included in the study, mRNA and protein SIRT6 levels were downregulated in NSCLC tissue versus normal lung tissue, and SIRT6 levels were inversely correlated with H3K56 levels. Positive rates of SIRT6 were significantly correlated with degree of cell differentiation, TNM stage, lymph node metastasis, overall survival and metastasis-free survival.

Conclusion: Downregulation of SIRT6 expression may promote NSCLC malignancy in the Chinese Han population. SIRT6 may be a potential therapeutic target in Chinese Han patients with NSCLC.

Keywords
SIRT6, non-small cell lung cancer, prognosis, malignancy

Date received: 8 June 2017; accepted: 1 December 2017

1Department of Respiratory Medicine, the People’s Hospital of Hai’an, Hai’an, Jiangsu, China
2Department of Cardiology, the People’s Hospital of Hai’an, Hai’an, Jiangsu, China

Corresponding author:
Bojin Zhu, Department of Respiratory Medicine, the People’s Hospital of Hai’an, 17 Zhongba Road, Hai’an 226600, Jiangsu, China.
Email: 41461986@qq.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Introduction

Globally, lung cancer is one of the most common causes of cancer-related death, with non-small cell lung cancer (NSCLC) accounting for about 80% of all lung cancer cases.\textsuperscript{1,2} Despite recent therapeutic advances in NSCLC, survival rates remain very low, with a 5-year overall survival in all stages of approximately 15\%.\textsuperscript{3} An understanding of genetic alterations and molecular mechanisms in the development of tumours is urgently required, with an aim to improving targeted therapy and prognosis in patients with NSCLC. Changes in glucose metabolic pathways are the common behaviours of tumour cells, laying the foundation for macromolecular synthesis in their growth and proliferation.\textsuperscript{4} Multiple studies have shown that down-regulated expression of pyruvate dehydrogenase kinase-1 and lactate dehydrogenase can inhibit tumour growth and proliferation,\textsuperscript{5–7} suggesting that changes of metabolism are important to the growth and proliferation of tumour cells.

Among the sirtuin (SIRT) family, the sirtuin 6 gene (\textit{SIRT6}), encodes nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase sirtuin-6 (SIRT6), an NAD\textsuperscript{+}-dependent histone acetylation enzyme that regulates multiple physiological processes, such as telomere maintenance, DNA repair, gene expression, aging and metabolism.\textsuperscript{8} Down regulation of \textit{SIRT6} has been shown in hepatocellular carcinoma, head and neck squamous cell carcinoma, pancreatic cancer and colorectal cancer, and \textit{SIRT6} has been demonstrated to regulate tumour metabolism, thus, \textit{SIRT6} is believed to be a tumour suppressor gene.\textsuperscript{9–11}

The relationship between \textit{SIRT6} expression levels and clinical features in Chinese Han patients with NSCLC remains unclear. Accordingly, the aim of the present study was to determine the lung tissue expression pattern of \textit{SIRT6} in Chinese Han patients with NSCLC, and to evaluate the relationships between \textit{SIRT6} expression and histone H3-acetyl K56 (H3K56), clinico-pathologic features, prognosis, and survival times following surgery and anticancer treatment.

Patients and methods

\textbf{Study population and tissue samples}

In this observational cohort study, Chinese Han patients with NSCLC who were admitted to the People’s Hospital of Hai’an, Jiangsu, China, for surgical resection of lung cancer tissue, between January 2011 and December 2012, were sequentially enrolled into the study. Patients who had received any cancer treatment prior to surgery were excluded from the study.

Cancer tissue and normal lung tissue (>5 cm away from the primary cancers) were collected from every patient during the surgical resection procedure. A proportion of paired (cancerous and noncancerous) fresh surgical specimens were randomly selected and immediately cooled and stored at –80°C for subsequent real-time reverse-transcription (RT)-polymerase chain reaction (PCR) and Western blot analyses. In addition, paired tissue specimens from all patients were soaked in 4% formalin solution for immunohistochemistry. Histological diagnosis and grade of differentiation were classified according to World Health Organization (WHO) classification guidelines,\textsuperscript{12} and cancer stage was defined as I, II, III or IV according to the pTNM staging system of the Union for International Cancer Control.\textsuperscript{13}

Patient’s clinical and demographic details were obtained by reviewing patient medical records. All patients received standard chemotherapy following surgery, according to the policies and procedures of the People’s Hospital of Hai’an, China.

The study was approved by the Institutional Review Board of the People’s
Hospital of Hai’an (IRB20110075), and all patients provided written informed consent.

**Real-time RT-PCR**

Total RNA was isolated from 100 mg of previously frozen cancerous and normal lung tissue specimens using 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. Prior to reverse transcription, the total RNA was quantified by spectrophotometric analysis at 260 nm and analysed as described in detail elsewhere. First, cDNA was synthesized using a Fermentas RevertAid™ First Strand cDNA synthesis kit (ThermoFisher Scientific, Burlington, Canada) and PTC-100 Thermal Cycler (MJ Research, Waltham, MA, USA) according to the manufacturer’s instructions. First strand cDNA was subsequently amplified by real-time quantitative PCR using Fast Start Universal SYBR Green Master Mix (Roche, Basel, Switzerland) in a Corbett RG-6000 PCR system (QIAGEN, Dusseldorf, Germany). Each reaction comprised the following: 1 μl of template cDNA, 10 μl master mix, 12 μl dH₂O and 1 μl (10 μmol/l) of the following sense and antisense primers: glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward 5’-GCAAGTTCAACGGCACAG-3’, reverse 5’-GCCAGTAGACTCCACGACA T-3’; SIRT6 forward 5’-CCCGGATCAACGGCTCTATC-3’, reverse 5’-GCCTTCACCTTTTTGGGGG -3’, made up to a final reaction volume of 25 μl. Quantitative real-time PCR was performed under the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 20 s and extension at 72°C for 20 s. The fold change in gene expression was evaluated using the comparative threshold cycle (Ct) method: $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct$ sample (normalised to GAPDH) – $\Delta Ct$ reference (normal tissue, also normalised to GAPDH).

**Western blot analysis**

Proteins were extracted from previously frozen cancerous and normal lung tissue from patients with NSCLC TNM stage I–II and patients with TNM stage III–IV. Protein was extracted using radioimmunoprecipitation assay (RIPA) buffer with 100 mM of phenylmethylsulphonyl fluoride as a protease inhibitor (Solarbio, Beijing, China) and analysed using protocols that have been published elsewhere. Briefly, equal amounts of protein were electrophoresed on a 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel and transferred onto nitrocellulose membranes (Millipore, Billerica, MA, USA). After blocking nonspecific binding with 5% fat-free milk for 30 min at room temperature, the membranes were incubated with rabbit anti-SIRT6 (1:500; Cat No. A7416; ABclonal Biotechnology, Woburn, MA, USA) or rabbit anti-H3K56 (1:1000; Cat No. ab71956; Abcam, Cambridge, MA, USA) at 4°C overnight. Membranes were then washed three times with Tris-buffered saline–Tween 20 (TBST; 1 l containing 3.02 g Tris-base, 8 g NaCl and 1 ml Tween 20), and incubated with 1:1000 dilution of horse radish peroxidase-conjugated goat anti-rabbit IgG (Cat No. ab6721; Abcam) for 2 h at room temperature. The membranes were then washed three times with TBST buffer and the immunoreactive signal was developed using Amersham enhanced chemiluminescence reagents (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK). Membranes were also incubated with rabbit polyclonal anti-β-actin antibody (1:5000; Cat No. ab8226; Abcam) at 4°C overnight, as a loading control. Optical densities were analysed using ImageMaster 2D Platinum software, version
Immunohistochemistry

Immunohistochemistry was performed with paired tissue specimens from all patients using a protocol described previously.17 Formalin-fixed tissue specimens were washed three times in PBS (pH 7.4), then successively soaked in 20% and 30% sucrose solution for dehydration, and frozen in optimal cutting temperature compound. Tissue sections (30 μm thick) were then prepared using a cryostat (Leica CM1900). Sections were incubated with 10% goat serum in phosphate buffered saline-Tween (PBST; 0.01 M sodium phosphate buffer, pH 7.4, containing 0.05% [v/v] Tween 20) for 1 h at room temperature to reduce nonspecific binding. The sections were then incubated with rabbit anti-SIRT6 (1:200; Cat No. ab191385; Abcam) at 4°C overnight, then washed three times in PBS (pH 7.4), followed by incubation with Invitrogen Alexa Fluor 568-conjugated goat anti-rabbit IgG (1:1000; Cat No. A-11036; ThermoFisher Scientific, Waltham, MA, USA). Cell nuclei were counter-stained with Hoechst 33342 (1:2000, ThermoFisher Scientific) for 30 min at room temperature. The sections were then observed using fluorescence microscopy at × 200 magnification.

Results from the immunostaining were interpreted independently by two expert pathologists who were blind to the clinical data. The comparative intensity of SIRT6 immunostaining was scored as follows: 0 (no signal), 1 (weak), 2 (moderate) and 3 (strong). At least five fields of view for each section were observed, and the percentage of positive cells (weak, moderate or strong signal) was calculated (0–100%). A semi-quantitative histopathology (H) score was obtained by multiplying the staining intensity score with the percentage score (0–300). An H-score higher than the median was considered as having positive expression.18

Statistical analyses

Data are presented as n and % prevalence, or as mean ± SD, and were analysed using SPSS software, version 21.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used to assess SIRT6 mRNA and protein levels and H3K56 protein levels in cancerous versus normal lung tissue specimens. Associations between SIRT6 expression and clinicopathologic features were analysed using Pearson’s χ²-test. Five-year overall survival and metastasis-free survival were the primary outcome measures, estimated using Kaplan-Meier survival curves, and differences in survival between SIRT6 positive and SIRT6 negative cases were compared using log-rank test. Statistical significance was set at P < 0.05.

Results

A total of 86 Chinese Han patients with NSCLC were included in the study: 58% male (50/86)/42% female (36/86), with an overall mean age of 57.1 years, range, 45–77 years (Table 1).

SIRT6 mRNA in lung tissue of Chinese Han patients with NSCLC

Real-time RT-PCR revealed that SIRT6 was expressed as mRNA in cancerous and normal lung tissue specimens from 20 Chinese Han patients with NSCLC. SIRT6 mRNA levels were found to be significantly lower in cancer tissue than in normal lung tissue, and significantly lower in cancerous tissue classified as TNM III–IV versus TNM I–II (both P < 0.05; Figure 1).
SIRT6 and H3K56 protein in lung tissue of Chinese Han patients with NSCLC

Western blot analyses showed that SIRT6 and H3K56 proteins were present in cancerous and normal lung tissue specimens from 10 patients with NSCLC TNM stage I–II and 10 patients with TNM stage III–IV. SIRT6 protein levels were found to be significantly lower in cancer tissue versus normal lung tissue, and significantly lower in cancer tissue classified as TNM III–IV versus TNM I–II (both \( P < 0.05 \); Figure 2). Conversely, H3K56 protein levels were significantly higher in cancer tissue than in normal lung tissue, and significantly higher in TNM III–IV cancer tissues than in TNM I–II cancer tissues (both \( P < 0.05 \); Figure 2).

Rate of SIRT6 positive samples in Chinese Han patients with NSCLC

The SIRT6 protein was mainly found in the nucleus of alveolar epithelial cells, and SIRT6 immunoreactive signal intensity was graded as zero, weak, moderate and strong. Strong staining was observed in normal lung tissue specimens, moderate staining was

| Characteristic                              | (n)   | SIRT6 expression | Statistical significance |
|---------------------------------------------|-------|------------------|-------------------------|
|                                             |       | Negative         | Positive                |
| All patients                                | 86    | 64 (74.42)       | 22 (25.58)              |
| Age                                         |       |                  |                         |
| \( \leq \) mean                             | 31    | 26 (83.87)       | 5 (16.13)               |
| \( > \) mean                                | 55    | 38 (69.09)       | 17 (30.91)              |
| Sex                                         |       |                  |                         |
| Male                                        | 50    | 35 (70.00)       | 15 (30.00)              |
| Female                                      | 36    | 29 (80.56)       | 7 (19.44)               |
| Smoking status                              |       |                  |                         |
| Current/former                             | 54    | 37 (68.52)       | 17 (31.48)              |
| Never                                       | 32    | 27 (84.38)       | 5 (15.62)               |
| Histological type                           |       |                  |                         |
| Adenocarcinoma                              | 49    | 33 (67.35)       | 16 (32.65)              |
| Squamous cell carcinoma                     | 37    | 31 (83.78)       | 6 (16.22)               |
| Degree of differentiation                   |       |                  |                         |
| Well                                        | 38    | 24 (63.16)       | 14 (36.84)              |
| Moderate/poor                               | 48    | 40 (83.33)       | 8 (16.67)               |
| Invasion of the visceral pleura             |       |                  |                         |
| No                                          | 46    | 32 (69.57)       | 14 (30.43)              |
| Yes                                         | 40    | 32 (80.00)       | 8 (20.00)               |
| TNM stage                                   |       |                  |                         |
| I–II                                        | 48    | 31 (64.58)       | 17 (35.42)              |
| III–IV                                      | 38    | 33 (86.84)       | 5 (13.16)               |
| Lymph node metastasis                       |       |                  |                         |
| No                                          | 37    | 23 (62.16)       | 14 (37.84)              |
| Yes                                         | 49    | 41 (83.67)       | 8 (16.33)               |

Data presented as \( n \) or \( n (%) \) prevalence.

NS, no statistically significant between-group difference (\( P > 0.05 \); Pearson’s \( \chi^2 \)-test).
found in TNM I–II tissue specimens and weak or zero staining was found in TNM III–IV tissue specimens (Figure 3a). The rate of SIRT6 positive tissue samples in cancer specimens was lower than in normal lung specimens and the rate of SIRT6 positive tissue samples in TNM III–IV cancer tissues was lower than in TNM I–II cancer tissues. ($P < 0.05$; Figure 3b).

### Correlation between SIRT6 expression and clinicopathologic factors

To determine whether SIRT6 expression levels were associated with clinicopathologic factors in Chinese Han patients with NSCLC, the rates of SIRT6 positive cases were assessed in relation to clinicopathologic data (Table 1). The rate of positive SIRT6 expression was found to be associated with degree of differentiation, TNM stage and lymph node metastasis ($P < 0.05$; Table 1). No statistically significant associations were observed between SIRT6 expression and other clinicopathologic factors.

### Relationship between SIRT6 expression and five-year overall survival

Median overall survival was significantly higher in SIRT6 positive patients (51 months, 95% CI 42.73, 59.27) versus SIRT6 negative patients (42 months [95% CI 37.87, 46.13]; $P < 0.05$ between the two groups). The 5-year overall survival rate was also significantly higher in SIRT6
Figure 3  Rate of positive nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase sirtuin-6 (SIRT6) expression, detected by immunohistochemistry, in lung tissue from 86 Chinese Han patients with non-small cell lung cancer (NSCLC): (a) Representative photomicrographs showing sirtuin 6 (SIRT6) protein signal intensity (graded as zero, weak, moderate and strong). Strong staining was found in normal lung specimens, moderate staining was found in TNM I–II specimens and weak or zero staining was found in TNM III–IV specimens. Magnification × 200; (b) Rate of positive SIRT6 expression was significantly lower in cancer specimens versus normal lung specimens, and the rate of positive SIRT6 expression decreased with tumour stage. Data presented as mean ± SD (*P < 0.05 versus normal lung specimens; **P < 0.05 versus TNM I–II specimens; One-way analysis of variance)
positive patients than in SIRT6 negative patients (27.27% versus 9.38%, respectively; \(P < 0.05\); Figure 4).

**Relationship between SIRT6 expression and metastasis-free survival**

Median metastasis-free survival was significantly higher in SIRT6 positive patients versus SIRT6 negative patients (45 months [95% CI 38.90, 51.10] versus 36 months [95% CI 33.90, 38.10], respectively; \(P < 0.05\); Figure 5).

**Discussion**

Despite improvements in surgery, external radiotherapy, chemotherapy and other treatment strategies, NSCLC remains one of the main causes of global cancer-related deaths.\(^1\,\!\!^2\) Thus, it is important to identify specific biomarkers associated with NSCLC, and develop novel anti-tumour therapies (such as molecular targeted therapy, immunotherapy and gene therapy). With the development of genetic technology, activation of oncogenes and inactivation of tumour suppressor genes have been reported to regulate the occurrence and development of lung cancer.\(^19\)

Sirtuin family members are thought to play key roles in carcinogenesis,\(^20\,\!\!^21\) and mutation of the SIRT6 chromosomal locus has been reported in a large number of acute myeloid leukemias,\(^22\) and liver, lung and breast cancers.\(^10\) Several preliminary studies have provided evidence to support the hypothesis that SIRT6 functions as a tumour suppressor gene.\(^9\,\!\!^11\) SIRT6 has been found to be down-regulated in various clinical specimens such as head and neck squamous cell carcinoma, pancreatic cancer, colorectal cancer, and hepatocellular carcinoma.\(^9\,\!\!^11\,\!\!^23\) In the current study, the
expression pattern of SIRT6 and correlations between SIRT6 and clinicopathologic characteristics were explored in Chinese Han patients with NSCLC: SIRT6 mRNA and protein levels were shown to be gradually decreased with tumour stage in this patient population. The rate of SIRT6 positive samples also gradually decreased with tumour stage in Chinese Han patients with NSCLC. The rate of positive SIRT6 samples was significantly correlated with degree of differentiation, TNM stage and lymph node metastasis. No significant associations were observed between SIRT6 expression and other NSCLC clinicopathologic factors.

Cells with an absence of SIRT6 protein exhibit abnormal chromatin modification, and global hyperacetylation of H3K56 that is correlated with tumorigenicity and tumour grade. In the present study, H3K56 expression in cancerous and normal lung tissue specimens from Chinese Han patients with NSCLC were detected by Western blot. H3K56 levels in cancerous tissue specimens was higher than in normal lung tissue specimens and the H3K56 level in TNM III–IV cancer tissues was higher than in TNM I–II cancer tissues. SIRT6 protein levels appeared to be inversely associated with H3K56 levels.

Survivin expression is reported to re-activate in most cancers and to be associated with poor prognosis. Survivin has been shown to block cancer cell apoptosis by inhibiting caspase3,7,9 and apoptosis inducing factor pathways. SIRT6 can repress survivin expression by modulating histone deacetylation or binding at the survivin promoter. Cytoplasmic SIRT6 expression is reported to be higher than nuclear SIRT6 expression in NSCLC primary cancer tissues, which has been associated with poor prognosis. In the present study, SIRT6 expression was mainly found in the nucleus. The survival time of SIRT6 positive patients with NSCLC was significantly longer than that of SIRT6 negative patients. At the same time, a low level of SIRT6 expression was associated with decreased metastasis-free survival time. Taken together, SIRT6 appears to play an anticancer role, possibly by promoting cancer cell apoptosis.

The results of the present study may be limited by several factors. First, the study included a relatively small cohort of patients with NSCLC and secondly, independent associations between SIRT6 and survival were not analysed. Further studies with a large number of patients are warranted to confirm the present results, and the mechanisms related to SIRT6 and cancer progression should be further studied.

In conclusion, the present findings demonstrate that SIRT6 is down-regulated in NSCLC and may be associated with malignant progression of NSCLC. These results suggest that SIRT6 should be further investigated as a potential therapeutic target for NSCLC.

Acknowledgements
Thanks are due to Bin Cao and Shulai Zhou for assistance with interpretation of the immunohistochemistry results.

Declaration of conflicting interests
The authors declare that there is no conflict of interest.

Funding
This work was supported by the Nantong Science and Technology Project (GJZ16116).

References
1. Siegel R, DeSantis C, Virgo K, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 2012; 62: 220–241.
2. Molina JR, Yang P, Cassivi SD, et al. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008; 83: 584–594.
3. Kamangar F, Dores GM and Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 2006; 24: 2137–2150.

4. Vander Heiden MG, Cantley LC and Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; 324: 1029–1033.

5. Bonnet S, Archer SL, Allalunis-Turner J, et al. A mitochondria-K\(^+\) channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* 2007; 11: 37–51.

6. Fantin VR, St-Pierre J and Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 2006; 9: 425–434.

7. Le A, Cooper CR, Gouw AM, et al., Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc Natl Acad Sci U S A* 2010; 107: 2037–2042.

8. Lerrer B, Gertler AA and Cohen HY. The complex role of SIRT6 in carcinogenesis. *Carcinogenesis* 2016; 37: 108–118.

9. Sebastian C, Zwaans BM, Silberman DM, et al. The histone deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism. *Cell* 2012; 151: 1185–1199.

10. Marquardt JU, Fischer K, Baus K, et al. Sirtuin-6-dependent genetic and epigenetic alterations are associated with poor clinical outcome in hepatocellular carcinoma patients. *Hepatology* 2013; 58: 1054–1064.

11. Lai CC, Lin PM, Lin SF, et al. Altered expression of SIRT gene family in head and neck squamous cell carcinoma. *Tumour Biol* 2013; 34: 1847–1854.

12. Travis WD. The 2015 WHO classification of lung tumors. *Pathologe* 2014; 35: 188.

13. Liszka L, Pajak J, Mrowiec S, et al. Discrepancies between two alternative staging systems (European Neuroendocrine Tumor Society 2006 and American Joint Committee on Cancer/Union for International Cancer Control 2010) of neuroendocrine neoplasms of the pancreas. *Pathol Res Pract* 2011; 207: 220–224.

14. Li H, Qin J, Jin G, et al. Overexpression of Lhx8 inhibits cell proliferation and induces cell cycle arrest in PC12 cell line. *In Vitro Cell Dev Biol Anim* 2015; 51: 329–335.

15. Zhou X, Jiang Y, Lu L, et al. MHC class II transactivator represses human IL-4 gene transcription by interruption of promoter binding with CBP/p300, STAT6 and NFAT1 via histone hypoacetylation. *Immunology* 2007; 122: 476–485.

16. Li Q, Qiao G, Ma J, et al. Downregulation of VEGF expression attenuates malignant biological behavior of C6 glioma stem cells. *Int J Oncol* 2014; 44: 1581–1588.

17. Yi X, Jin G, Zhang X, et al. Cortical endo- genic neural regeneration of adult rat after traumatic brain injury. *PLoS One* 2013; 8: e70306.

18. Nomura S, Suzuki Y, Takahashi R, et al. Dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) as a novel marker in T1 high-grade and T2 bladder cancer patients receiving neoadjuvant chemotherapy. *BMC Urol* 2015; 15: 53.

19. Zhang Y, Zhao Y, Sun S, et al. Overexpression of MicroRNA-221 is associated with poor prognosis in non-small cell lung cancer patients. *Tumour Biol* 2016; 37: 10155–10160.

20. Bosch-Presegue L and Vaquero A. The dual role of sirtuins in cancer. *Genes Cancer* 2011; 2: 648–662.

21. Carafa V, Nebbio A and Altucci L. Sirtuins and disease: the road ahead. *Front Pharmacol* 2012; 3: 4.

22. Mahlknecht U, Ho AD and Voelter-Mahlknecht S. Chromosomal organization and fluorescence in situ hybridization of the human Sirtuin 6 gene. *Int J Oncol* 2006; 28: 447–456.

23. Wysocka J, Swigut T, Milne TA, et al. WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methyl- ation and vertebrate development. *Cell* 2005; 121: 859–872.

24. Das C, Lucia MS, Hansen KC, et al. CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* 2009; 459: 113–117.
25. Tamm I, Wang Y, Sausville E, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 1998; 58: 5315–5320.

26. Altieri DC and Marchisio PC. Survivin apoptosis: an interloper between cell death and cell proliferation in cancer. Lab Invest 1999; 79: 1327–1333.

27. O’Connor DS, Grossman D, Plescia J, et al. Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc Natl Acad Sci U S A 2000; 97: 13103–13107.

28. Shin S, Sung BJ, Cho YS, et al. An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7. Biochemistry 2001; 40: 1117–1123.

29. Mesri M, Wall NR, Li J, et al. Cancer gene therapy using a survivin mutant adenovirus. J Clin Invest 2001; 108: 981–990.

30. Liu T, Brouha B and Grossman D. Rapid induction of mitochondrial events and caspase-independent apoptosis in Survivin-targeted melanoma cells. Oncogene 2004; 23: 39–48.

31. Azuma Y, Yokobori T, Mogi A, et al. SIRT6 expression is associated with poor prognosis and chemosensitivity in patients with non-small cell lung cancer. J Surg Oncol 2015; 112: 231–237.