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Original Article

Low SIRT3 expression contributes to tumor progression, development and poor prognosis in human pancreatic carcinoma

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ABSTRACT

SIRT3, an important mitochondrial protein, may act as either an oncogene or tumor suppressor depending on the tumor-type. The aim of this study was to investigate the expression of SIRT3 in pancreatic carcinoma (PC) and its clinical association in PC patients. Immunohistochemistry was adopted to investigate the expression of SIRT3 in cancer and corrresponding adjacent non-cancer tissues across 79 patients with PC. The log-rank test and Cox hazard model were used to estimate the relationship between SIRT3 expression and prognosis. The staining results revealed that SIRT3 negative expression was more common in cancer tissues than in adjacent non-cancer tissues ($P < 0.001$). Chi-square tests indicated that the expression of SIRT3 correlated with T status ($p < 0.001$) and tumor stage ($p = 0.013$). Kaplan-Meier analysis showed that negative SIRT3 expression is linked to a poor prognosis in PC patients. Multivariate analysis identified SIRT3 expression as an independent predictor for PC outcome both in the whole cohort and several subgroups of PC patients. Our results indicate that down-regulated SIRT3 may contribute to tumor progression and gloomy prognosis in PC patients and may sever as a novel prognostic marker.

1. Introduction

Pancreatic carcinoma (PC) is one of the most lethal malignancies and the fourth leading cause of cancer-related deaths in the United States [1]. Due to its high propensity for locoregional invasion and early development of distant metastases, only 10%–15% of patients present with early-stage disease that permits curative surgery [2,3]. More than 90% of patients who have received a diagnosis of PC die from the disease [4], and the 5-year survival rates of PC patients usually do not exceed 5% [5]. This poor prognosis is mainly attributed to its aggressive growth, difficulty of early diagnosis, limited effective therapies, and lack of biomarker as a prognostic indicator of PC patients. Hence, there is an urgent need for the identification of novel prognostic molecular factors to improve the survival rates for this disease.

The sirtuin family, which functions either as nicotinamide adenine dinucleotide(NAD^+)-dependent deacetylase [6] or as ADP-ribosyl transferases [7], play roles in many physiology and pathophysiology conditions, including regulation of oxidative stress, increased genomic stability, cell survival, cell division, aging, metabolism and carcinogenesis [8–12]. In humans as well as in all mammalian this family is composed by seven different homologous proteins, namely SIRT1 ~ -SIRT7 [13]. Of the 7 mammalian sirtuins, SIRT3 is thought to be the primary mitochondrial deacetylase and has now been shown to regulate many aspects of mitochondrial function [14]. To our knowledge, mitochondrial dysfunction is a common feature of tumors. Thus, SIRT3 has been a major focus of study on understanding cancer.

Researchers have demonstrated that SIRT3(-/-) mouse embryonic fibroblasts exhibited abnormal mitochondrial physiology and SIRT3(-/-) mouse were more likely to develop ER/PR positive mammary tumors; what's more, human breast cancer specimens exhibited reduced SIRT3 levels [15]. It has also been reported that the expression of SIRT3 was decreased in hepatocellular carcinoma [16,17], lung cancer [18] and gastric cancer [19,20], etc. All these findings support a tumor suppressor role for SIRT3 in cancers. In contrast, other reports indicated that SIRT3 may act as an oncogene [21–23]. Based on the discrepancy in current literatures, it's of immerse importance to identify the expression status of SIRT3 and to further investigate its clinical

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significance in different type of tumors.

In this retrospective study, we tried to clarify the clinical importance of SIRT3 in PC. We examined the expression pattern of SIRT3 and explored the relationship between SIRT3 expression and clinicopathologic parameters, including overall survival. Our data showed that the expression of SIRT3 was correlated with favorable prognosis of PC patients.

2. Materials and methods

2.1. Patients and tissue samples

79 samples along with complete clinical and pathological data were collected from PC patients who underwent surgical resection at Fuzhou General Hospital of Nanjing Military Command, Fuzhou, China, during May 2005 to December 2014. The study cohort consisted of 54 males and 25 females. Ages ranged from 27 to 77 (median 57). 57 cases were located in pancreatic head, 7 in the body, 7 in the tail and 8 in combined locations. Postsurgical survival data were available for all 79 patients and the following data were extracted: age, sex, tumor location, tumor size, degree of differentiation, TNM stage, conditions of lymph node involvement and infiltration of perineural. Tumor stage was defined according to the 7th edition of AJCC cancer staging manual. Formalin-fixed paraffin-embedded cancer and its non-cancer tissue counterpart were used. Before surgery, all the patients did not receive any kinds of therapeutic regimen, such as chemotherapy, radiotherapy or targeted therapy. Informed consent was obtained from all patients or from patients' guardians and the study was approved by the Ethics Committee of Fuzhou General Hospital of Nanjing Military Command.

2.2. Immunohistochemical staining

79 pairs of formalin-fixed, paraffin-embedded pancreatic cancer and its adjacent normal tissues were cut into 4um slides. Slides were dewaxed through descending grades of ethanol concentrations and then washed in phosphatebuffered saline (PBS). Slides were immersed in antigen-unmasking solution and boiled in a pressure cooker for 20mins. After that, endogenous peroxide was removed by 3% hydrogen peroxide in PBS to reduce nonspecific background staining. Slides were then incubated with rabbit anti-human monoclonal antibody to SIRT3 (1:500) (#2627; Cell Signaling Technology, Danvers, MA, USA) for 2 h and then washed in PBS. After that, these slides followed the standard procedure for the two-step immunostaining kit (Elivision kit-0015; Fuzhou Maixin Biological Technology Ltd, Fujian, China). The staining was visualized with 3, 30-diamino-benzidine tetrahydrochloride, counterstained with Mayer hematoxylin, air-dried, and mounted.

2.3. Evaluation of staining results

All slides were independently evaluated by YY and LS who were blinded to the clinicopathologic and survival data. The brown cytoplasm coloration was considered as the positive signal. According to previous suggested [24], SIRT3 scores were divided into two groups: negative and positive. Non-immune rabbit serum diluted at the same concentration with monoclonal antibody to SIRT3 was served as negative control.

2.4. Follow up

Follow-up was performed for all the patients through telephone call and/or outpatient clinic. The follow-up time ranged from 1 month to 60 months, during this period 73 patients have died.

2.5. Statistical analysis

SPSS 18.0 software (SPSS Inc, Chicago, IL, USA) was used to perform all the analysis. We utilized Chi-square test to analysis the relationship between SIRT3 expression and the clinicopathological characteristics of patients. McNemar's test was adopted to compare SIRT3 expression between cancer and adjacent non-cancer tissues. Besides that, we used Pearson's correlation analysis to further investigate the relationship between them. Kaplan-Meier method was applied to plot survival curve and the difference between these two curves was verified by log-rank test. Multivariate Cox regression (Proportional hazard model) analysis was conducted to determine independent prognostic factors. Two sides P value less than 0.05 was deemed as statistically significant.

3. Results

3.1. Expression of SIRT3 and its association with the clinicopathological features in PC

It was shown that SIRT3 was mainly located in the cytoplasm in the cells (Fig. 1). Among the 79 PC specimens, SIRT3 negative expression rate in cancer tissues was 60.7% (48/79), while in adjacent non-cancer tissues this ratio low to 26.6% (29/79). SIRT3 negative expression was more common in cancer tissues than in adjacent non-cancer tissues ($P < 0.001$, McNemar's test). The correlation between SIRT3 expression in cancer tissues and that in adjacent non-cancer tissues were also statistical significant ($p = 0.001$, Pearson's correlation analysis). The relationship between SIRT3 expression and various clinicopathologic parameters is showed in Table 1. The expression of SIRT3 protein correlated with T status ($p < 0.001$) and tumor stage ($p = 0.013$). No significant relationship with other clinicopathologic parameters was found. Of note, SIRT3 expression in adjacent non-cancer tissues was not related to the above clinicopathologic parameters (data not shown).

3.2. Association between SIRT3 protein and OS in the whole cohort of PC

We further investigated the association between SIRT3 protein and OS through Kaplan-Meier survival analysis. Survival data were available for all the 79 patients. The average OS time for patients in SIRT3 positive expression group was 18.81 ± 3.38 months, however it was only 8.06 ± 0.80 months for patients in SIRT3 negative expression group. Patients in SIRT3 negative expression group had poorer OS ($P = 0.001$) than patients in SIRT3 positive expression group, according to Kaplan-Meier survival analysis and log-rank test (Fig. 2).

3.3. Univariate and multivariate analyses of prognostic factors for PC patients

Cox survival analysis was used to analyze the prognostic factors for PC patients. According to univariate analysis, cell grade, T status, N status, peri-neural invasion, tumor stage and SIRT3 expression were identified to be correlated with OS. In multivariate cox regression, SIRT3 negative expression was proved to be a significant independent prognostic factor for poor OS in PC patients (HR = 1.83, 95% CI 1.00–3.33, P = 0.049) (Table 2). Other independent prognostic factor was cell grade ($p = 0.016$).

3.4. Influences of SIRT3 expression on overall survival in subset of PC

Based on the 9 clinicopathologic parameters evaluated above, the whole patients were divided into 18 subsets. Univariate analysis indicated SIRT3 expression as a significant predictor for overall survival in 11 subsets of PC patients, such as Males, Age < 57, Age > 57, T1-2, N0, Tumor size(≤2 cm), PNI present or absent, Tumor stage(I–II), Cell grade(well-moderate) and Pancreatic head (Supplementary Fig.