European Review for Medical and Pharmacological Sciences 2017; 21: 5399-5401

A novel screening test for esophageal squamous cell carcinoma: sirtuin-3

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Abstract. – OBJECTIVE: Human sirtuin-3, a protein involved in the mediation of tumors, has been shown to be present in malignancies. The goal of this study was to measure serum sirtuin-3 levels in esophageal squamous cancer cells and to determine whether sirtuin-3 may possess predictive value in advanced cases of esophageal squamous cell carcinoma (ESCC).

PATIENTS AND METHODS: A total of 130 ESCC patients and 50 healthy control subjects participated to the study. Serum sirtuin-3 levels for all 180 subjects were measured using enzyme-linked immunosorbent assay (ELISA).

RESULTS: Median sirtuin-3 levels were significantly higher in patients with ESCC than in the control subjects.

CONCLUSIONS: The presence of considerably elevated levels of sirtuin-3, could be a powerful mediator of advanced ESCC in ESCC patients, suggests that sirtuin-3 may be a useful indicator of the disease.

Key Words:
Sirtuin-3, Screening, Esophageal cancer ESCC.

Introduction

Despite recent advances in the treatment of esophageal squamous cell carcinoma (ESCC)[1,2], this disease remains lethally malignant, primarily because presentation occurs at a late stage, when the tumor is no longer responsive to surgical resection. The two- and five-year actuarial survival rates of ESCC patients are 48% and 14%, respectively. Novel approaches to the treatment of ESCC, such as neoadjuvant chemotherapy and chemoradiotherapy, present no clear advantage over surgical resection alone[1,2].

The poor prognosis for this cancer is largely due to delayed diagnosis. Various tumor markers have been employed in the diagnosis of ESCC[3,4]. Analysis of serum sirtuin-3 activity in ESCC patients could be useful in determining whether sirtuin-3 activity can serve as a useful tumor marker in this pathology, which in turn may hold promise for the early diagnosis of esophageal cancer.

In this study, we used enzyme-linked immunosorbent assay (ELISA) to measure serum sirtuin-3 levels in patients with ESCC, in order to assess the clinical value of sirtuin-3 in the diagnosis of ESCC.

Patients and Methods

Patients

The study involved a total of 180 participants, a study group consisting of 130 ESCC patients (age range 28-73 years, median age 57.31 ± 5.84 years; 45 women and 85 men) and 50 healthy control subjects (age range 28-70 years, median age 56.22 ± 6.17 years; 30 women and 20 men). Peripheral blood samples from all participants were collected at the Yuzuncu Yil University, Departments of General Surgery, Thoracic Surgery, and Gastroenterology (Van, Turkey).

All patients with histologically proven ESCC, as confirmed either by endoscopic biopsy or surgery, who were available for follow-up, were included in the study.

All data obtained were kept confidential and used only after receiving formal written consent. The study was approved by the Ethics Committee of Yuzuncu Yil University Hospital.

Collection of Serum Samples

In each case, 3 mL of peripheral blood collected from each subject was quickly transferred to an ordinary tube and allowed to settle at room temperature (24-25°C) for 20-25 min. Next, 1 mL

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Table I. The demographic variables and Sirtuin-3 levels of the controls group and patients with ESCC.

	Controls (n = 50)	Patients (n =130)	*p*-value
Age years	56.22 ± 6.17	57.31 ± 5.84	> 0.05
Min-Max	(28-70)	(28-73	
Gender (M/F)	(20/30)	(85/45)	
Total sirtuin-3 (ng/mL)	1.1 ± 0.3	7.5 ± 1.2	< 0.001
Min-Max	(0.3-2)	(6-8)	

of supernatant was aspirated and transferred to a clean 1 mL centrifuge tube, and the following steps were completed within 1 h (at room temperature) or 2 h (at 4°C): centrifugation at 1500 rpm for 20 min at 4°C, collection of the supernatant, and storage at -80°C for future use.

Biochemical Analysis

Serum levels were analyzed in duplicate with the inclusion of two quality control samples for every run. Levels of sirtuin-3 were measured using commercially available sandwich ELISA (enzyme-linked immunosorbent assay) (Human Sirtuin-3 ELISA kit, code: MBS091264 MyBiosource, San Diego, CA, USA). Sensitivity was 0.1 ng/mL (range: 0.25-8 ng/mL). Intra- and inter-assay coefficients of variability (CV) were both less than 15% [CV (%) = SD/mean × 100].

Statistical Analysis

The Shapiro-Wilk normality test and One Sample Kolmogorov-Smirnov test were performed, and a histogram, Q-Q plot, and box plot were drawn. The data presented are mean, standard deviation, median, minimum, maximum, and frequency, the last given as a percentage. The sirtuin-3 variable, which exhibited normal distribution between the two groups, was analyzed using the independent samples *t*-test, while age, which was not normally distributed, was analyzed using the Mann-Whitney U test. The variable of gender was evaluated using a x^2 test with the Yates continuity correction. The cut-off for significance was a *p*-value less than 0.05, based on a bi-directional hypothesis. Analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., Armonk, NY, USA).

Results

Table I presents demographic and clinical-pathological data as well as test results for all study participants.

Equal variances for age were assumed to be 0.251, and the two groups did not significantly differ in age (p = 0.573; Figure 1). The gender breakdown was also similar in both the patient and control groups (p = 0.728). The mean serum sirtuin-3 level was 7.5 ± 1.2 ng/mL for ESCC patients and 1.1 ± 0.3 ng/mL for healthy subjects. Serum sirtuin-3 levels were significantly higher in patients with ESCC than in the control group (p < 0.001; Table I and Figure 2).

Discussion

The present study found serum sirtuin-3 levels to be significantly elevated in patients with ESCC compared with healthy subjects.

Because sirtuin-3 appears to be a factor in carcinogenesis, its inhibitors/activators may have potential therapeutic efficacy[6]. Sirtuin-3 is linked to a form of metabolic reprogramming termed the "Warburg effect" in cancer cells[7] and has been shown to play a role in angiogenesis, tissue invasion, and metastasis[8]. A study found elevated sirtuin-3 levels in oral squamous cell carcinoma (OSCC) cell lines and human OSCC tissue samples[9]. Sirtuin-3 expression was also recently reported to have been downregulated in 4 paired hepatocellular tissues[10].

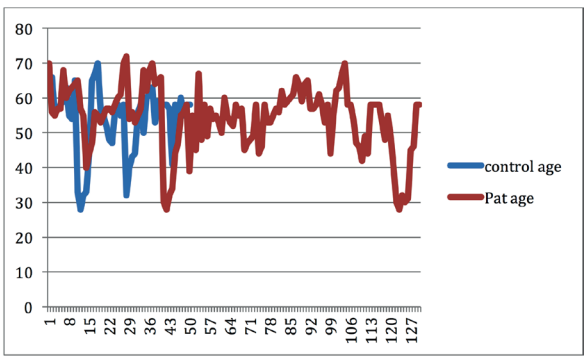

Figure 1. The histogram of the age of the all subjects.

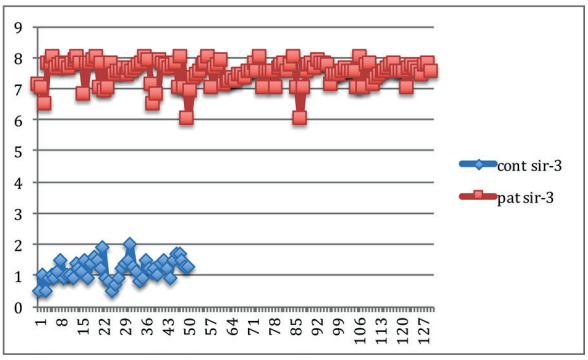

Figure 2. The histogram of the sirtuin-3 levels of the all subjects.

Data thus far have demonstrated that sirtuin-3 exhibits dichotomous functions, either as a tumor promoter or suppressor[11]. Evidence supporting the role of sirtuin-3 in tumor promotion was provided by a study, which found that sirtuin-3 prevented the growth arrest of bladder cancer cells[12]. In another study[13], the persistence of high sirtuin-3 levels could have been supported by the ability of sirtuin-3 to induce apoptosis.

The dichotomous role of sirtuin-3 in cancer, in both the promotion and suppression of tumors, highlights the importance of further investigation of this subject.

Conclusions

The present study has provided evidence that sirtuin-3 is found in elevated concentrations in ESCC patients, particularly in those with disseminated cases. Furthermore, it has demonstrated that measuring serum sirtuin-3 levels may be of value in determining the postoperative survival of ESCC patients. However, the nature of the mechanism of sirtuin-3 in the progression of ESCC is still imperfectly understood, and thus this topic warrants further examination.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Law S, Kwong DL, Kwok KF, Wong KH, Chu KM, Sham JS, Wong J. Improvement in treatment results and long-term survival of patients with esophageal cancer: impact of chemoradiation and change in treatment strategy. Ann Surg 2003; 238: 339-348.
2) Zaw S, Wong J. Current management of esophageal cancer. J Gastrointest Surg 2005; 9: 291-310.
3) Shimada H, Kitabayashi H, Nabeya Y, Okazumi S, Matsubara H, Funami Y, Miyazawa Y, Shiratori T, Uno T, Itoh H, Ochiai T. Treatment response and prognosis of patients after recurrence of esophageal cancer. Surgery 2003; 133: 24-31.
4) Shimada H, Nabeya Y, Okazumi S, Matsubara H, Miyazawa Y, Shiratori T, Hayashi H, Gunji Y, Ochiai T. Prognostic significance of CYFRA 21-1 in patients with esophageal squamous cell carcinoma. J Am Coll Surg 2003; 196: 573-578.
5) Shi T, Wang F, Stieren E, Tong Q. SIRT3, a mito-chondrial sirtuin deacetylase, regulates mito-chondrial function and thermogenesis in brown adipocytes. J Biol Chem 2005; 280: 13560-13567.
6) Bruzzone S, Parenti MD, Grozio A, Ballestrero A, Bauer I, Del Rio A. Rejuvenating sirtuins: the rise of a new family of cancer drug targets. Curr Pharm Des 2013; 19: 614-623.
7) Bell EL, Guarente L. The sir3 divining rod points to oxidative stress. Mol Cell 2011; 42: 561-568.
8) Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
9) Alhazzazi TY, Kamarajan P, Joo N, Huang JY, Verdin E, D'Silva NJ, Kapila YL. Sirtuin-3 a novel potentiak therapeutic target for oral cancer. Cancer 2011; 117: 1670-1678.
10) Zhang YY, Zhou LM. Sirt3 inhibits hepatocellular carcinoma cell growth through reducing Mdm2-mediated p53 degradation. Biochem Biophys Res Commun 2012; 423: 26-31.
11) Alhazzazi TY, Kamarajan P, Verdin E, Kapila YL. SIRT3 and cancer: tumor promoter or suppressor? Biochim Biophys Acta 2011; 1816: 80-88.
12) Li S, Banck M, Mujtaba S, Zhou MM, Sugrue MM, Walsh MJ. p53-induced growth arrest is regulated by the mitochondrial sirT3 deacetylase. PLoS One 2010: 5; e10486.
13) Allison SJ, Milner J. SIRT3 is pro-apoptotic and participates in distinct basal apoptotic pathways. Cell Cycle 2007; 6: 2669-2677.