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# Clinicopathological and predictive significance of SIRT1 and peroxisome proliferator-activated receptor gamma in esophageal squamous cell carcinoma: The correlation with EGFR and Survivin

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ABSTRACT

SIRT1 (silent mating type information regulation 2 homolog 1) is an enzyme that deacetylates proteins that contributes to cell survival and angiogenesis. Peroxisome proliferator-activated receptor Y (PPAR Y) is a member of the nuclear steroid hormone receptor superfamily and regulates cell apoptosis and proliferation. The functional roles of SIRT1 and PPAR Y in tumor progression remain controversy. This study aims to investigate the roles of SIRT1 and PPAR Y in esophageal squamous cell carcinoma (ESCC), as well as correlation with expression of EGFR and Survivin. Here, we analyzed the protein expression of SIRT1 and PPAR Y in tumor microarray with ESCC and its associations with clinicopathological parameters and overall survival. Both SIRT1 and PPAR Y were highly expressed in tumor tissues comparing with non-cancerous epithelium. High expression of SIRT1 was positively correlated with advanced TNM stage and poor outcome, while high expression of PPAR Y was positively related with tumor grading, not with patients' prognosis. In addition, the high expression of SIRT1 was positively correlated with overexpression of EGFR, not related with PPAR Y or Survivin expression status. These data suggests SIRT1 may serve as a predictor of poor prognosis in ESCC, and its mediated tumor-promoting role might be associated with the overexpression of EGFR protein in ESCC.

## 1. Introduction

Cancer of the esophagus is one of the most lethal malignancies of the gastrointestinal tract. The esophageal squamous cell carcinoma (ESCC) is the dominant histological type of esophageal cancer in China. Although multidiscipline approaches with neoadjuvant chemotherapy, radiochemotherapy and surgical therapy developed in the past two decades, the overall survival of ESCC remains low [1,2]. Therefore, it is urgent need to investigate molecular oncogenesis and prognostic markers of ESCC to achieve a tailored multimodality effective treatment so as to improve patients' outcome with ESCC.

Sirtuin 1 (SIRT1), also known as NAD-dependent deacetylase sirtuin-1, is an enzyme that deacetylates proteins that contributes to cellular regulation. SIRT1 is the most extensively identified member of the sirtuin family, and has involved in deacetylating not only histones, but also many nonhistone proteins including p53, forkhead class O transcription factor (FOXO), NF-κB, retinoblastoma protein (Rb), E2F1, mismatch repair gene MLH1, PTEN, androgen receptor, which are indicated in cell growth, apoptosis and tumorigenesis [3–7]. The functional role of SIRT1 in cancer progression is controversial. Although SIRT1 has been suggested to play a tumor-suppressor role, recent evidence indicated its oncogenic properties. SIRT1 has been shown to promote survival and inhibit apoptosis of tumor cells [8,9]. Furthermore, high SIRT1 expression is reported in pancreatic ductal adenocarcinoma [10], lung adenocarcinoma [11], liver [12] and gastrointestinal cancer [13]and predicted a poor prognosis in these tumors.

Peroxisome proliferator-activated receptor Y (PPAR Y) is a member of the nuclear steroid hormone receptor superfamily and participates in adipose differentiation and fat metabolism. Although previous studies suggested activation of PPAR Y inhibited cell growth of esophageal squamous cell carcinoma [14], other studies showed expression of PPAR Y was correlated with cell differentiation of esophageal cancer [15]. In addition, Takahashi reported PPAR Y antagonists inhibited invasive properties of esophageal cancer cells [16]. However, little is known about the prognostic significance of SIRT1 and PPAR Y and their correlation in esophageal squamous cell carcinoma. Therefore, here, we

*Abbreviations:* SIRT1, silent mating type information regulation 2 homolog 1; PPAR Y, peroxisome proliferator-activated receptor Y; EGFR, epidermal growth factor receptor; ESCC, esophageal squamous cell carcinoma

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examined the expression of SIRT1 and PPAR Y, as well as related proteins such as EGFR and Survivin, and their relationships to clinicopathological features and prognosis of patients with esophageal squamous cell carcinoma.

## 2. Materials and methods

### 2.1. Case selection and tissue samples

95 paraffin-embedded tumor samples of ESCC and 95 paracancerous normal tissues were obtained from the biobank in National Engineering Research Center for Biochip at Shanghai. All tissues specimens were obtained following with patient informed consent for retention and analysis of their tissue for research purpose, and Ethical approval for the study was obtained from the Ethical Committee of Biobank Center related hospitals. The diagnosis of ESCC was determined by pathologists and a database was established for clinical parameters such as age, gender, tumor size, histological grading, pathological stage and Overall survival (OS). The stage was updated according to current American Joint Committee on cancer (AJCC) guidelines. The surgical time was from January 2006 to October 2008 with follow-up time for 5 years. During the following-up period, 64 patients died of cancer recurrence and metastasis, 31 patients were still alive.

### 2.2. Tissue microarray construction

Tissue microarrays (TMA) were constructed using diameter of 1.5-mm cores. The representative of tumor tissues and the corresponding tissues adjacent to carcinoma were selected by the pathologists. TMA blocks were constructed using an automated tissue arrayer (Beecher Instruments, Sun Prarie, WI). The array blocks were cut into five-micron sections, and one section was stained with hematoxylin-eosin to verify the presence of tumor cells.

### 2.3. Immunohistochemistry and evaluation

Immunohistochemical method (Envision ™ two-step method) was used to detect the protein expression of SIRT1, PPAR Y, EGFR and Survivin in tissue microarray of ESCC. Tissues sections were deparaffinized with xylene, rehydrated with graded alcohol. Antigen retrieval was performed by high pressure repair in 0.1 M citrate buffer (pH 6.0) for 10 min. After quenching of endogenous peroxidase activity, SIRT1 antibody (dilution 5 μg/ml, Abcam Inc. MA, USA), PPAR Y antibody (dilution 1:100, LifeSpan BioSciences, Inc), EGFR antibody (dilution 1:100, Abcam Inc. MA, USA), Survivin antibody (dilution 1:100, Abcam Inc. MA, USA) were incubated at 4 ˚C overnight, then followed by incubation with HRP-Conjugated anti-rabbit and mouse secondary antibody (Dako, Denmark) for 45 min at room temperature, the sections were developed in 0.05% diaminobenziding and counter-stained with hematoxylin before dehydration and mounting. The positive staining of SIRT1 and PPAR Y was located in the nucleus. The positive staining of EGFR was mainly located in cell membrane and less in cytoplasm. The positive staining of Survivin was mainly located in the nucleus. The degree and percentage of immunoreactivity were evaluated independently by two pathologists without knowledge of the clinical status and outcome data. The staining scores were calculated as the positive percentage multiplying the staining intensity for each biological marker. The positive percentage was scored as “1” (1%–25%), “2” (26%–50%), “3” (51%–75%) and “4” (76%–100%). Intensity was scored as “0‘(negative), “1’ (weak), ‘2’ (moderate), and “3” (strong). The cases with total scores more than six for SIRT1 and PPAR Y were classified as high expression, the rests as low expression [10]. For EGFR, the cases of at least 20% tumor cells with complete moderate or strong membrane staining were considered as overexpression or positive expression, otherwise as low or negative expression [17]. For Survivin, the cases with more than 20% positive cells of moderate or strong staining were defined as high expression, otherwise as low expression [18]. Cases with discrepancies were reevaluated until a consensus was reached. Positive and negative controls were used during each staining run to identify any possible problems with immunohistochemistry.

### 2.4. Statistical analysis

The analyses were performed using the Statistical Package for Social Sciences, Version 16.0, for Windows (SPSS, Chicago, IL). The chi-square test was used to evaluate the association between each index and the clinicopathological characteristics. Kaplan-Meier analysis was performed for survival curves, and statistical significance was assessed using the log-rank test. Overall survival was defined as the time from surgery to the date of death. Multivariate analysis using the Cox proportional hazard regression model was used to evaluate an independent prognostic factor of overall survival. The statistical significance of correlation between SIRT1, PPAR Y, EGFR and Survivin expression level in ESCC tissues was estimated by Spearman’s rank correlation analysis. Statistical significance was defined as $P < 0.05$.

## 3. Results

### 3.1. Patient characteristics

Patients with primary esophagus squamous cell carcinoma consist of 74 (77.9%) male and 21 (22.1%) female, with an average age of 65 (range, 45–79). Based on tumor differentiation degree, there are 44 (46.3%) patients with low-grade tumor, and 51 (53.7%) patients with high-grade tumor. There are 62 (65.3%) patients whose maximum diameter is more than 5 centimeter. The 95 cases of patients were classified according to the 7th Edition of TNM system of the American Joint Committee on Cancer. There are 24 (25.3%) patients in stage I, 31 (32.6%) in stage II, 40 (42.1%) in stage III (Table 1).

**Table 1**
Relationship between the expression of SIRT1 and PPAR Y and clinicopathological parameters in esophageal squamous cell carcinoma.

| Clinicopathologic parameters | Total (n = 95) | SIRT1 | | P -value | PPAR Y | | P -value |
|---|---|---|---|---|---|---|---|
| | | High | low | | High | low | |
| Gender | | | | | | | |
| Male | 74 | 49 | 25 | 0.969 | 37 | 37 | 0.082 |
| Female | 21 | 14 | 7 | | 15 | 6 | |
| Age | | | | | | | |
| ≥60 | 51 | 32 | 19 | 0.428 | 29 | 22 | 0.654 |
| < 60 | 44 | 31 | 13 | | 23 | 21 | |
| Tumor size | | | | | | | |
| ≥ 5cm | 62 | 42 | 20 | 0.687 | 31 | 31 | 0.204 |
| < 5cm | 33 | 21 | 12 | | 21 | 12 | |
| Tumor grade | | | | | | | |
| Low grade | 44 | 30 | 14 | 0.721 | 29 | 15 | 0.042* |
| High grade | 51 | 33 | 18 | | 23 | 28 | |
| TNM stage | | | | | | | |
| I + II | 55 | 31 | 24 | 0.016* | 26 | 29 | 0.087 |
| III | 40 | 32 | 8 | | 26 | 14 | |
| EGFR | | | | | | | |
| Positive | 59 | 44 | 15 | 0.029* | 34 | 25 | 0.469 |
| Negative | 36 | 19 | 17 | | 18 | 18 | |
| Survivin | | | | | | | |
| High | 54 | 36 | 18 | 0.934 | 28 | 26 | 0.517 |
| Low | 41 | 27 | 14 | | 24 | 17 | |

*represents statistical significance.