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The clinicopathological significance of SIRT1 expression in colon cancer: An immunohistochemical study and meta-analysis

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ABSTRACT

Objective: The aim of this study was to determine the clinicopathological significance and potential prognostic role of SIRT1 expression in colorectal cancer (CRC) using immunohistochemistry and meta-analysis.
Methods: Immunohistochemistry was performed on 265 archival paraffin-embedded human CRC specimens to investigate the correlation between SIRT1 expression and clinicopathological characteristics, including patient survival. To elucidate the potential prognostic value of SIRT1 expression, a meta-analysis was performed using data on 2132 patients from eight eligible studies.
Results: SIRT1 was highly expressed in 24.5% of the 265 CRC specimens analyzed. High SIRT1 expression correlated with vascular invasion ($P = 0.041$). High SIRT1 expression also significantly correlated with expression of SNAI ($P = 0.001$), but not E-cadherin ($P = 0.958$). However, there was no significant correlation between SIRT1 expression and other clinicopathological parameters. High SIRT1 expression in the CRC specimens significantly correlated with a worse overall survival rate, independent of SNAI expression. However, based on the meta-analysis, high SIRT1 expression was not significantly correlated with overall survival rates [hazard ratio (HR) 1.111, 95% confidential interval (CI) 0.799–1.544].
Conclusion: In our retrospective study, high SIRT1 expression significantly correlated with vascular invasion and a worse prognosis. However, because the results from the meta-analysis differed the retrospective arm of our study, additional cumulative studies are needed to determine the prognostic value of SIRT1 in CRC.

1. Introduction

Silent information regulator 2 (SIR2), which plays important roles in gene transcription, cell metabolism, and stress response, is known as an anti-aging gene of yeast that encodes NAD^+-dependent histone deacetylase [1,2]. Silent mating type information regulation 2 homolog 1 (SIRT1) is a mammalian homolog gene of SIR2 that is located in the cell nucleus [1,2]. SIRT1 deacetylates histones and non-histone proteins such as p53, nuclear factor-kappa B (NF-κB), and forkhead box (FOX) proteins. To affect various functions, including cell metabolism and cell survival, SIRT1 regulates various transcription factors, including NF-κB and FOXO [3]. In addition, SIRT1 is frequently expressed in various malignant tumors, including colorectal cancer (CRC), breast cancer, hepatocellular cancer, prostate cancer, ovarian cancer, and bladder cancer [4]. SIRT1 may act as a tumor promoter that represses apoptosis and promotes metastasis in various malignant tumors [4]. Other studies report contradictory roles of SIRT1 in malignant tumors. For example, SIRT1 acts as a tumor suppressor, including promoting apoptosis [4]. SIRT1 also promotes epithelial-mesenchymal transition (EMT), which accelerates cancer invasion and metastasis by reducing the expression of epithelial markers like E-cadherin and occludin and increases expression of mesenchymal markers like vimentin, fibronectin, and SNAI [5]. However, other studies show that SIRT1 is overexpressed in CRC and that its overexpression correlates with aggressive features [6–8]. Therefore, the prognostic value of SIRT1 in CRC is controversial.

In the current study, we examined the correlation between SIRT1 expression in CRC and clinicopathological characteristics and patient survival. The correlation between SIRT1 and EMT markers relating to the role of SIRT1 in CRC invasiveness and metastasis was also evaluated. Finally, a meta-analysis was performed in effort to elucidate any prognostic value of SIRT1 in CRC.

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2. Material and methods

2.1. Patients and tissue array methods

The files of 265 patients who had undergone surgical resection of CRC at the Eulji Medical Center, between January 1, 2001 and December 31, 2010, were analyzed. We reviewed medical charts, pathological records, and glass slides in order to assess clinicopathological characteristics such as age, gender, tumor size, tumor location, tumor differentiation, vascular, lymphatic, and perineural invasion, depth of tumor, lymph node metastasis, metastatic lymph node ratio, distant metastasis and pathologic tumor node metastatic (pTNM) stages.. This protocol was reviewed and approved by the Institutional Review Board of Eulji University Hospital (Approval No. EMC 2017-10-013). Five array blocks containing a total of 265 resected CRC tissue cores obtained from patients were prepared. Briefly, tissue cores were taken from individual paraffin-embedded CRC tissues (donor blocks) and arranged in recipient paraffin blocks using a trephine apparatus, as previously described [9]. A core was chosen from each case for analysis. An adequate case was defined as a tumor occupying more than 10% of the core area. Each block contained internal controls consisting of non-neoplastic colon tissue. Clinical outcomes were followed from the date of surgery to either the date of death or recurrence, resulting in a follow-up period ranging from 0 to 60 months.

2.2. Immunohistochemical staining and evaluation

Sections 4 μm in thickness were cut from each tissue-array block, deparaffinized, and dehydrated using a routine xylene-alcohol series. For antigen retrieval, sections were treated with 0.01 M citrate buffer (pH 6.0) for 5 min in a microwave oven followed by treatment with 3% H_2O_2 to quench endogenous peroxidase. Sections were treated with normal serum of the host animal of the secondary antibody to block nonspecific binding. Sections were then incubated with anti-SIRT1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), anti-SNAI (Santa Cruz Biotechnology), and anti-E-cadherin (Santa Cruz Biotechnology). Immunohistochemical stainings were conducted following a compact polymer method using a VENTANA benchmark XT autostainer (, Ventana Medical Systems Inc., Tucson, Arizona). Visualization was performed by treatment with OPTIVIEW universal 3,3′-diaminobenzidine kit (Ventana Medical Systems Inc.). To confirm the reaction specificity of the antibody, a negative control stain without primary antibody was performed. All immunostained sections were lightly counterstained with Mayer's hematoxylin.

The immunohistochemistry results were evaluated by two independent researchers. SIRT1 and SNAI showed immunoreactivity in the nucleus of tumor cells, whereas E-cadherin showed immunoreactivity in the cell membrane. The intensity of protein expression in the immunohistochemically stained samples was scored from 0 to 3 (0 = negative; 1 = weak; 2 = moderate; and 3 = strong). The percentage of positively stained cells was based on a scoring system from 0 to 4 (1 = 0–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%). An immunoreactive score (IRS) was then calculated by multiplying the scores of staining intensities and the percentage of positively stained cells [10]. Based on the IRS, SIRT1, SNAI, and E-cadherin staining patterns were classified as low (IRS: 0 to 4) or high (IRS: 6 to 12).

2.3. Published studies search and selection criteria for meta-analysis

Relevant articles were obtained by searching the PubMed and Embase databases up to February 28, 2018. These databases were searched using the following key words: 'SIRT1′ and 'survival, prognosis, mortality, or fatality'. The titles and abstracts of all searched articles were screened for exclusion. Review articles were also screened to find additional eligible studies. Articles were included if the study was performed in human colorectal cancers, and if there was information about the correlation between SIRT1 expression and survival rate. Articles were excluded if they were case reports or non-original articles, or if the article was not written in English.

2.4. Data extraction

Data from all eligible studies were extracted by two independent authors. The included data were extracted from each of the eligible studies [5,8,11–15]. We collected the first author's name, year of publication, study location, number of patients analyzed, and information for the correlation between SIRT1 expression and survival rate. For quantitative aggregation of survival results, the correlation between SIRT1 expression and survival rate was analyzed according to the hazard ratio (HR) using one of three methods. In studies not quoting the HR or its confidence interval (CI), these variables were calculated from the presented data using the HR point estimate, log-rank statistic or its *P*-value, and the O–E statistic (difference between the number of observed and expected events) or its variance. If those data were unavailable, HR was estimated using the total number of events, number of patients at risk in each group, and the log-rank statistic or its *P*-value. Finally, if the only useful data were in the form of graphical representations of survival distributions, survival rates were extracted at specified times to reconstruct the HR estimate and its variance under the assumption that patients were censored at a constant rate during the time intervals [16]. The published survival curves were read independently by two authors in order to reduce reading variability. The HRs were then combined into an overall HR using Peto's method [17].

2.5. Statistical analysis

Statistical analyses were performed using SPSS version 22.0 software (SPSS, Chicago, IL, USA). The significance of the correlation between SIRT1 expression and the clinicopathological characteristics was determined by either χ^2 test or Fisher's exact test (two-sided). The comparisons between SIRT1 expression and age, tumor size, or metastatic lymph node ratio were analyzed using the two-tailed Student's *t*-test Survival curves were estimated using the Kaplan-Meier product-limit method, and differences between the survival curves were determined to be significant based on the log-rank test. In addition, for the multivariate analysis, including SNAI expression, cox-regression test was conducted. Results were considered statistically significant for $P < 0.05$. In addition, for meta-analysis, all data were analyzed using the Comprehensive Meta-Analysis software package (Biostat, Engelwood, NJ, USA). The meta-analysis was performed by fixed-effects and random-effects models. Because eligible studies used various population and evaluation criteria and, the values pooled using the random effects model were utilized for interpretation. Subsequently, a study showing results of an estimated HR > 1 without a 95% confidence interval (CI) overlapping 1 implied poor survival with SIRT1 expression. For assessment of publication bias, Begg's funnel plot and Egger's test were performed. The results were considered statistically significant when $P < 0.05$.

3. Results

3.1. Correlation between SIRT1 expression and clinicopathological characteristics of CRC

High SIRT1 expression was observed in 65 of the 265 CRC specimens analyzed (24.5%). Fig. 1 shows representative results from the immunohistochemical analysis of SIRT1 expression in the CRC tissues. High SIRT1 expression was correlated with vascular invasion ($P = 0.041$; Table 1). However, high expression levels of SIRT1 was not correlated with other clinicopathological characteristics, including age, gender, tumor size, tumor location, tumor differentiation, lymphatic