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Original article

Differential expression of the sirtuin family in renal cell carcinoma: Aspects of carcinogenesis and prognostic significance

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Abstract

Objectives: Sirtuins (1–7) are evolutionarily conserved NAD-dependent deacetylases that play an important role in carcinogenesis. However, their role in renal cell carcinoma (RCC) remains unclear. The objective of the present study was to examine the role of SIRTs in RCC carcinogenesis and prognosis.

Materials and methods: Paraffin-embedded specimens from 102 patients who underwent extirpative renal surgeries for renal masses between January 2004 and December 2010 were examined. SIRT expression was compared between RCC and adjacent normal kidney tissues by immunohistochemical staining. Survival differences and cancer-specific survival were analyzed with the Kaplan-Meier log-rank test and univariate and multivariate Cox regression analyses, respectively.

Results: SIRT1, SIRT3, and SIRT6 expression was significantly lower in RCC than in normal tissues ($P = 0.001$, $P = 0.006$, and $P = 0.033$, respectively), whereas the expression of other SIRT proteins did not differ significantly between the 2 tissues. SIRT3 expression was significantly associated with longer cancer-specific survival (HR $= 0.133$, $P = 0.047$), after adjusting for age, T stage, Fuhrman grade, Karnofsky performance status, and distant metastases. Kaplan-Meier analysis showed that patients with high-SIRT3 expression had relatively better survival than those with low-SIRT3 expression ($P = 0.046$, log-rank test).

Conclusions: Our results provide preliminary evidence suggesting that SIRT1, SIRT3, and SIRT6 function as tumor suppressors in RCC. In particular, SIRT3 seems to have a favorable influence on the survival of patients with clear cell RCC.

Keywords: Sirtuin; SIRT; Renal cell carcinoma; RCC; Immunohistochemistry

1. Introduction

Renal cell carcinoma (RCC) accounts for approximately 90% of all kidney malignancies, which in turn account for 2% to 3% of all adult cancers worldwide [1]. RCC is the most lethal urological tumor and the sixth leading cause of cancer-associated mortality in Western countries [1]. To date, much effort has been made to identify a genetic regulator with diagnostic and prognostic potential in RCC. However, the precise role of the sirtuin (SIRT) family of genes is not well reported.

SIRTs constitute a highly conserved gene family found in organisms ranging from bacteria to humans that play key

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roles in cellular apoptosis, aging, and resistance to metabolic stress [2,3]. Seven mammalian SIRTs (SIRT1–7) have been identified, and deregulated expression of these genes is involved in the development of various malignancies [4,5]. The best-characterized member of this family is SIRT1, which is predominantly located in the nucleus [6]. Recent reports suggest a carcinogenic function of SIRT1 in lung, breast, and colon cancers; however, its role as a putative tumor suppressor in different cancer types was also suggested [7–9]. Less is known about SIRT2. SIRT2, the primary cytoplasmic SIRT, is downregulated in human gliomas, breast cancer, and hepatocellular carcinoma [10,11]. SIRT3 to SIRT5 are mitochondrial SIRTs that link aging to energy metabolism [12]. SIRT3 is overexpressed in oral cancer and lymph node-positive breast cancer [13,14]. However, data indicate a suppressive role of SIRT3 in hepatocellular carcinoma [15], lung adenocarcinoma [16], and gastric cancer [17]. SIRT4 plays an important role in insulin regulation, and its activity is downregulated by calorie restriction [18]. The precise role of SIRT4 and SIRT5 in cancer development remains unclear. The remaining 2 SIRTs, SIRT6 and SIRT7, are localized in the nucleus and cytoplasm [6,19]. SIRT6 is a candidate tumor suppressor, as its expression is significantly decreased in hepatocellular carcinoma [20]. SIRT7 is a positive regulator of RNA polymerase I transcription and is required for cell proliferation and survival [21]. SIRT7 is also upregulated in breast and thyroid cancers [13,22].

These results indicate that altered expression of SIRTs may contribute to the development of cancer and other clinicopathological conditions. However, few studies have examined the role of the SIRT family in RCC. The present study explored the potential role of the SIRT family in RCC by evaluating the expression of SIRT proteins and their relationship to the clinicopathological features of patients with RCC.

2. Materials and methods

2.1. Study population

The clinical, radiologic, and pathologic records of 119 consecutive patients who underwent extirpative surgery for renal tumors between January 2004 and December 2010 were assessed in accordance with the Institutional Review Board guidelines of Gyeongsang National University Hospital. None of the patients had received chemotherapy or radiation therapy before surgical tumor resection. Clinicopathologic data such as sex, age, Karnofsky performance status, Fuhrman grade, and tumor-node-metastasis stage were, retrospectively, collected from medical records. To assess cancer-specific survival (CSS), the survival status and cause of death were investigated using the National Cancer Registry Database and institutional electronic medical records. In survival analyses, only patients with the clear cell type of RCC were included and those with a positive surgical margin were excluded.

2.2. Pathologic data analysis

Pathologic outcomes were reviewed by 2 genitourinary pathologists. Primary RCC was assigned tumor-node-metastasis stage and grade in accordance with the American Joint Committee on Cancer (AJCC) Staging Manual (seventh edition) and the Fuhrman grade system, respectively. Fuhrman grades I and II were considered to be low grade, and Fuhrman grades III and IV were classified as high grade.

2.3. Tissue microarray

Resected tumor samples were fixed overnight in 20% buffered neutral formalin. The samples were grossly examined, dissected, and embedded in paraffin blocks. Representative portions of tumor and adjacent normal tissues (>10 mm from the tumor) were selected by microscopic examination of hematoxylin and eosin stained sections from each specimen. One 2.0 mm core of tumor per case and one 2.0 mm core of normal tissue were arrayed.

2.4. Immunohistochemical analysis

Immunohistochemical (IHC) staining was performed on tumor tissues and adjacent normal tissues of RCC patients. Tissue microarray blocks were cut into 4 μm slices for IHC staining. After deparaffinization and rehydration, slides were incubated in 3% hydrogen peroxide for 10 minute to block endogenous peroxidase activity, which resulted in nonspecific background staining. Sections were then heated for 20 minute in 10 mM citrate buffer (pH = 6.0) in a microwave oven (700 W). After blocking endogenous peroxidase activity with a peroxidase quenching solution, the sections were incubated overnight at 4°C with anti-SIRT antibodies. The following monoclonal or polyclonal antibodies against SIRT proteins were used: SIRT1 (clone H-95; Santa Cruz Biotechnology), SIRT2 (clone H-300; Santa Cruz Biotechnology), SIRT3 (clone C73E3; Cell Signaling Technology), SIRT4 to 7 (ab90485, ab78982, ab118487, and ab78977, respectively; all from Abcam).

2.5. Scoring of immunoreactivity

The expression levels of SIRT1 to 7 were semiquantitatively scored by assessing the intensity of staining (0, no staining; 1, mild staining; 2, moderate staining; and 3, strong staining) and the percentage of positively stained cells (0, $<30\%$; 1, 30–49%; 2, 50–69%; and 3, $\geq 70\%$). The sum index was obtained by combining the staining intensity and percentage scores. Scoring was repetitively performed by 2 pathologists who were blinded to the clinical information. The degree of expression was evaluated by calculating