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Original article

Expression of Leptin and Sirtuin-1 is associated with poor prognosis in patients with osteosarcoma

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ABSTRACT

Sirtuin-1 (SIRT1) is a downstream target of Leptin, and its inhibition promotes p53-mediated apoptosis. This study aimed to evaluate the expression and prognostic significance of Leptin and SIRT1 in osteosarcoma. Leptin and SIRT1 levels in osteosarcoma samples from 89 patients were evaluated by immunohistochemical staining. The correlations between Leptin and SIRT1 expression with clinical parameters were analyzed by Spearman's test and Pearson's chi-squared test. Prognostic factors were identified by Univariate and multivariate Cox regression analysis. We found that Leptin and SIRT1 expression was low in 23.6% and 20.2%; moderate in 25.8% and 24.7%; and high in 50.5% and 55.1% of patients with osteosarcoma, respectively. Both Leptin and SIRT1 expression were significantly associated with the Enneking stage, distant metastasis and neo-adjuvant chemotherapy. Leptin expression and SIRT1 expression were significantly correlated and they were significantly associated with shorter overall survival. Among osteosarcoma patients who received neo-adjuvant chemotherapy, both Leptin and SIRT1 expression were significantly associated with overall survival of osteosarcoma patients in univariate analysis, but only SIRT1 expression was significantly associated with overall survival of osteosarcoma patients in multivariate analysis. In conclusion, Leptin and SIRT1 expressions are significantly associated with shorter overall survival of osteosarcoma patients, and SIRT1 expression is a significant independent prognostic indicator in patients with osteosarcoma.

Introduction

Osteosarcoma (OS) is a locally aggressive malignant tumor of mesenchymal origin, and patients with OS are susceptible to early systemic metastases [1]. Although the long-term outcome for patients who undergo surgery for high-grade OS has improved with the addition of systemic chemotherapy, prognosis of OS remains unsatisfactory [2]. OS is characterized by local invasion and early lung metastasis, resulting in a 5-year survival rate of 20% in patients with metastases [3,4]. Better prognostic indicators are urgently needed for patients with refractory OS who develop chemoresistance.

Leptin is a crucial regulator of energy metabolism and has gained increasing attention as a potential anticancer drug target. Leptin regulates body weight homeostasis via negative feedback on hypothalamic nuclei to modulate food intake behaviors [5]. In addition, Leptin mediates epithelial–mesenchymal transition in several cancers such as lung, breast, and colon cancer, resulting in poor survival and unfavorable clinical outcomes [6,7].

Sirtuin-1 (SIRT1), a conserved NAD-dependent protein deacetylase that regulates cellular lifespan and encourages carcinogenesis, is a downstream target of Leptin [8]. Overexpression of SIRT1 has been shown to be essential for the growth and survival of cancer cells [9]. SIRT1 overexpression has been documented in prostate, breast and gastric cancers, and in lymphoma and sarcomas [10–14]. However, in patients with OS, associations of Leptin or SIRT1 expression with clinical outcomes have not yet been demonstrated. In the present study, we evaluated the expression patterns of Leptin and SIRT1 in different patient-derived OS tissue samples. We also investigated the clinical significance of Leptin and SIRT1 expression status in patients with OS, and in those who underwent neoadjuvant chemotherapy.

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Methods

Ethics statement

This study complied with the Declaration of Helsinki and was approved by Ethics Committees of the Fourth Hospital of Hebei Medical University. All patients provided written informed consent.

Patients and samples

The present study evaluated paraffin-embedded specimens (89 OS and 10 osteochondroma for use as non-cancerous controls) that were collected between 2002 and 2010 from patients who visited the Fourth Hospital of Hebei Medical University. Clinical and histopathological characteristics and follow-up and survival information were available for all patients, and were collected retrospectively from medical records. Radiotherapy was not administered prior to surgery in any patient. Patients' data were grouped according to the age, gender, tumor size (<5 cm vs. ≥5 cm), histologic cancer type according to the World Health Organization classification, neoadjuvant chemotherapy administration, the presence of distant metastasis, and Enneking stage (I, II, or III).

Immunohistochemical staining and scoring

For immunohistochemical analysis, a tissue microarray was produced using 3.0-mm diameter tumor cores with 1 core per case. Antigen retrieval was performed by microwaving the array in sodium citrate buffer for 12 min. Immunohistochemical staining was performed as previously described [15,16]. Primary antibodies including rabbit anti-human leptin (1:100; Abcam, ab16227) and rabbit anti-human SIRT1antibodies (1:50; Santa Cruz Biotechnology; clone H-300). The stained sections were analyzed under an optical microscope by 2 pathologists (YD and YL) who were blinded to patient data. Leptin staining was cytoplasmic, whereas SIRT1 staining was primarily nuclear. The mean number of immunopositive cells was determined in 5 fields-of-view at ×400 magnification. The staining percentage was graded as follows: 0, <10%; 1, 10–30%; 2, 31–50%; 3, 51–75%; 4, >75%. Intensity was graded according to the degree of staining color depth as: 0, no staining; 1, buff; 2, darker buff; 3, tan. To obtain final immunohistochemical scores, the product of staining percentage and grade was used and staining was described as low (score 0, +); moderate (score 1–8, ++), or high (9–12 high, +++) [15–17].

Statistical analysis

The end of follow-up was defined as the date of death or last contact up to January 2014. Overall survival was defined as the time from the date of diagnosis to the date of last contact or death. Patients who were alive at last contact were censored for overall survival analysis. Associations between Leptin and SIRT1 expression levels and potential prognostic factors were analyzed using the χ^2 test. For correlation analysis, the Spearman-rho test was used to compare histological and clinical variables. Univariate and multivariate analysis were conducted using Cox proportional hazards regression analysis. The Kaplan–Meier method was used to illustrate the overall survival further. All statistical analyses were conducted using SPSS software (version 21.0), and $p < 0.05$ was considered significant.

Results

Patient characteristics

Patients' characteristics are shown in Table 1. The mean patient age was 22 years (range, 9–43 years), and the median overall survival was 11 months (range, 3–83 months).

Table 1
Clinicopathological variables and the expression status of leptin and SIRT1.

Characteristics			Leptin				SIRT1			
			Low (%)	Moderate (%)	High (%)	$p(\chi^2)/p$ (Spearman)	Low (%)	Moderate (%)	High (%)	$p(\chi^2)/p$ (Spearman)
Gender	Female	38	8 (21.0)	15 (39.5)	15 (39.5)	0.038/0.272	5 (13.2)	11 (28.9)	22 (57.9)	0.331/0.400
	Male	51	13 (25.5)	8 (15.7)	30 (58.8)		13 (25.5)	11 (21.6)	27 (52.9)	
Age	≥20 years	39	9 (23.1)	9 (23.1)	21 (53.8)	0.835/0.668	7 (17.9)	7 (17.9)	25 (64.1)	0.287/0.190
	<20 years	50	12 (24.0)	14 (28.0)	24 (48.0)		11 (22.0)	15 (30.0)	24 (48.0)	
Tumor size	<5 cm	41	7 (17.0)	12 (29.3)	22 (53.7)	0.395/0.363	8 (19.5)	11 (26.8)	22 (53.7)	0.913/0.895
	≥5 cm	48	14 (29.2)	11 (22.9)	23 (47.9)		10 (20.8)	11 (22.9)	27 (56.3)	
Histologic grade	Well differentiated	25	8 (32.0)	6 (24.0)	11 (44.0)	0.084/0.335	8 (32.0)	6 (24.0)	11 (44.0)	0.368/0.325
	Moderately differentiated	36	5 (13.9)	14 (38.9)	17 (47.2)		4 (11.1)	10 (27.8)	22 (61.1)	
	Poorly differentiated	28	8 (28.6)	3 (10.7)	17 (60.7)		6 (21.4)	6 (21.4)	16 (57.1)	
Neoadjuvant chemotherapy	Yes	53	19 (35.8)	17 (32.1)	17 (32.1)	<0.001/<0.001	16 (30.2)	17 (32.1)	20 (37.7)	<0.001/<0.001
	No	36	2 (5.5)	6 (16.7)	28 (77.8)		2 (5.6)	5 (13.9)	29 (80.6)	
Distant metastasis	Yes	56	4 (7.1)	16 (28.6)	36 (64.3)	<0.001/<0.001	5 (8.9)	12 (21.4)	39 (69.6)	<0.001/<0.001
	No	33	17 (51.5)	7 (21.2)	9 (27.3)		13 (39.4)	10 (30.3)	10 (30.3)	
Enneking stage	I	12	10 (83.4)	1 (8.3)	1 (8.3)	<0.001/<0.001	9 (75.0)	2 (16.7)	1 (8.3)	<0.001/<0.001
	II	22	7 (31.8)	8 (36.4)	7 (31.8)		3 (13.6)	9 (40.9)	10 (45.5)	
	III	55	4 (7.3)	14 (25.4)	37 (67.3)		6 (10.9)	11 (20.0)	38 (69.1)	
SIRT1 expression	Low	18	12 (66.7)	1 (5.6)	5 (27.8)	<0.001/<0.001	–	–	–	–
	Moderate	22	7 (31.8)	9 (40.9)	6 (27.3)		–	–	–	
	High	49	2 (4.1)	13 (26.5)	34 (69.4)		–	–	–	

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