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Correlation between survivin expression and locoregional control in cervical squamous cell carcinomas treated with radiation therapy

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Abstract

Objective. Survivin is a member of the inhibitors of apoptosis and has been implicated in both the regulation of cell division and the suppression of apoptosis. Over-expression of cytoplasmic survivin correlates with an unfavorable prognosis in many malignant tumors. However, the prognostic value of nuclear survivin expression is still equivocal. Here, we investigated the prognostic value of survivin expression in cervical cancer treated with radiation therapy.

Methods. Tissue sections were obtained from 72 patients with cervical squamous cell carcinoma treated with radiation therapy alone. Survivin expression levels were determined by immunohistochemical staining and evaluated for cell positivity. The correlation between survivin expression and clinical outcome endpoints including cause-specific survival and local control were evaluated.

Results. A total of 14% (10/72) of tissue specimens had greater than 5% nucleus positivity, while 47% (34/72) had greater than 50% cytoplasmic positivity. Local control rate of the cytoplasmic survivin-negative tumors was 94%, significantly higher than the 76% of the positive tumors ($p=0.046$). Local control rate of the nuclear survivin-positive and cytoplasmic survivin-negative patients was 95%, significantly higher than the 74% of the other patients ($p=0.02$). In contrast, no significant correlation was noted between survivin expression and disease-free survival.

Conclusions. The cytoplasmic survivin expression alone and the combination of nuclear and cytoplasmic expression were suggested to be predictors for local control in patients with cervical squamous cell carcinoma treated with radiation therapy alone.

Keywords: Cervix; Radiation therapy; Survivin; Squamous cell; Cytoplasmic

Introduction

Survivin, a member of the inhibitors of apoptosis, is expressed in the G2/M phases of the cell cycle, and has been implicated in both regulating cell division and in suppressing apoptosis [1]. Survivin is expressed almost exclusively in tumor cells, and is absent in most normal adult differentiated tissue [2]. Hence, survivin is considered to represent a potential new target for apoptosis-based therapy in cancer and lymphoma [2].

Expression of survivin in tumor cells has been reported to be associated with tumor progression [3], degree of invasiveness and malignancy [4], and resistance to radiation or anti-cancer drugs [5]. Over-expression of survivin has been associated with an unfavorable prognosis in malignant tumors [4,6–8]. However, the implications of survivin over-expression in tumor cell nuclei remain unclear, and may differ from that in the cytoplasm. Further, to our knowledge, there are only a few report describing correlations between survivin expression and prognosis in cervical cancer [9].

In this study, we investigate the prognostic value of survivin expression in tumor cell nuclei and cytoplasm in patients with cervical cancer treated with radiation therapy.

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Materials and methods

Patients and specimens

Seventy-two consecutive patients with squamous cell carcinoma of the uterine cervix who received radiation therapy at the Research Center Hospital of Charged Particle Therapy, National Institute of Radiological Sciences, Chiba, Japan, between 1988 and 1993 were investigated. Patients with distant metastases and those for whom paraffin-embedded tumor samples were not available were excluded. Mean age of patients was 64.2±11.7 years (range, 37–83 years). Clinical stage and histological classification were based on the criteria established by the International Federation of Gynecology and Obstetrics (FIGO) [10] and the World Health Organization (WHO) [11], respectively. The number of patients with stage I, II, III, and IVa disease was 1, 19, 50 and 2, respectively. Median follow-up was 60.5 months, ranging from 4 to 168 months. Five patients died of intercurrent diseases and 5 were lost to follow-up within 2 years. The remaining 62 patients were followed for a minimum of 2 years, until recurrence or metastasis was found, or death, whichever occurred first. Biopsy specimens were excised from the cervical tumors before radiation therapy, and subsequently fixed with 10% formalin for 24 h and embedded in paraffin.

Radiation therapy protocol

Patients were treated with a combination of conventional external pelvic irradiation and high-dose rate intracavitary irradiation. Details of the treatment protocol have been previously reported [12]. Briefly, patients were treated with external whole pelvic irradiation with anteroposterior and posteroanterior parallel opposing ports, with a dose of 1.8 Gray (Gy) per fraction, five times per week, to a total dose of 30.6 Gy. This was followed by a central shielding pelvic field irradiation, with a dose of 2 Gy per fraction, five times per week, to a total dose of 20 Gy. In addition to central shielding irradiation, these patients also received intracavitary irradiation. For the high-dose rate treatment, intracavitary irradiation was in principle performed with ^{60}Co or ^{192}Ir sources with four insertions (1 per week) and fraction doses of 5.0–6.0 Gy at Point A. Chemotherapy was not used as an initial treatment.

Immunohistochemical protocol and evaluation

Immunohistochemical studies were performed to detect the expression of survivin using polyclonal anti-Survivin antibody (Novus Biologicals, CO, U.S.A.). Sections were deparaffinized and dehydrated. Microwave unmasking of antigens was performed for 75 min in Dako Target Retrieval Solution (Dako, CA, U.S.A.) at 93.0°C followed by cooling for 20 min. Endogenous peroxide was subsequently blocked with peroxidase-blocking solution (Dako) for 15 min, followed by washing for 5 min with phosphate-buffered saline (PBS). The sections were incubated overnight at 4.0°C with anti-Survivin antibody diluted by antibody diluent (Dako) at 1:300. They were then washed three times in PBS for 5 min each and incubated for 30 min with labeled-polymer conjugated second antibody; envision+ kit (Dako). They were washed and developed with 3,3′-diaminobenzidine tetrahydrochloride for 2 min, lightly counterstained with hematoxylin, dehydrated and mounted. A known positive control (breast cancer) section was similarly stained. For a negative control, the incubation step was done with antibody diluent instead of primary antibody. All specimens were examined pathologically by a single pathologist (K.O.) who was blinded to clinical data. The nuclei and cytoplasms of more than 1000 tumor cells were evaluated in each specimen. Nuclear positive staining was defined when more than 5% of the tumor cells were stained in each section [14] and cytoplasmic positive staining was defined when more than 50% of the tumor cells were stained in each section. A cut off value of cytoplasmic staining was established as a positive result.

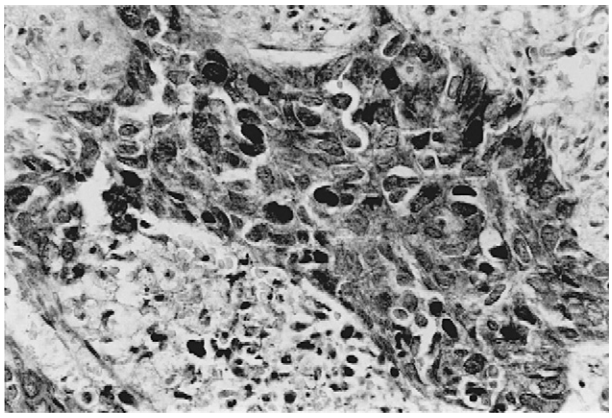

Fig. 1. Squamous cell carcinoma cells positive for survivin in nucleus and cytoplasm; survivin immunostaining (original magnification ×200).

Table 1
Distribution of the 72 patients according to combined nuclear and cytoplasm survivin expressions

Survivin expression	Number of patients
Nuclear(−) cytoplasm(−)	31 (43%)
Nuclear(+) cytoplasm(−)	7 (10%)
Nuclear(−) cytoplasm(+)	31 (43%)
Nuclear(+) cytoplasm(+)	3 (4%)
Total	72

Statistical analysis

The Kaplan–Meier products-limit method was used to estimate the probability of cause-specific disease-free survival and local control rate, and their difference was estimated with the log-rank test. The Fisher exact test was used to estimate the difference in local control rate between nucleus survivin-positive and -negative tumors only. The Fisher exact test was used to evaluate associations among survivin expression, clinical parameters and prognosis. The data of the multivariate analysis for local control were assessed with the Cox proportional multivariate analysis. All analyses were performed with StatView (Version 5.0, SAS Institute Inc, NC, U.S.A.).

Results

Squamous cell carcinomas showed either or both nuclear and cytoplasmic positive findings for survivin (Fig. 1). A total of 14% (10/72) of the tissue specimens demonstrated greater than 5% nuclear positivity for survivin, and 47% (34/72) demonstrated greater than 50% cytoplasmic survivin positivity (Table 1). Thirty-one tumors showed nuclear survivin-negative and cytoplasmic survivin-positive findings. Table 2 shows the nuclear and cytoplasmic survivin-positive rates according to disease stage: the positive rate of cytoplasmic survivin was significantly higher in advanced tumors (stage I

Table 2
Nuclear and cytoplasmic survivin positive rate according to clinical stage

Clinical stage	(pt)	N(+)	(pt)	C(+)	(pt)		N(−) & C(+)	(pt)
I	(1)	0%	(0)	0%	(0)		0%	(0)
II	(19)	21%	(4)	21%	(4)		21%	(4)
						p=0.007		
III	(50)	12%	(6)	56%	(28)		50%	(25)
IVa	(2)	0%	(0)	100%	(2)		100%	(2)

N: nuclear survivin expression, C: cytoplasmic survivin expression, (−): negative, (+): positive, (pt): number of patients.