Original Articles

Concomitant expression of HER2 and HIF-1α is a predictor of poor prognosis in uterine cervical carcinoma treated with concurrent chemoradiotherapy: prospective analysis (KGROG0501)

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Summary

Background: In previously reported retrospective analyses of uterine cervical carcinoma cases, HER2 was correlated with poor radiation sensitivity and poor treatment outcomes and HIF-1α was found to be an indicator of poor prognosis. To date, no prospective studies have been performed to evaluate the radiation sensitivity and treatment outcomes of patients with uterine cervical carcinoma relative to HER2 and HIF-1α expressions. We conducted a prospective evaluation of HER2 and HIF-1α in cases of locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy. *Methods:* Between June 2005 and April 2008, 25 patients with locally advanced uterine cervical carcinoma were registered in this study, KGROG0501. Their clinical stages were Ib2/IIb/IIIb/IVa in 1/2/22/1 cases, respectively. Nineteen cases had squamous cell carcinoma and six had adenocarcinoma. HER2 expression and HIF-1α expression were analyzed using an immunohistochemical kit on pretreatment biopsied specimens. HIF-1α expression was studied using another commercial immunohistochemical kit on pretreatment biopsied specimens. The survival rates were compared between patients with and without positive HER2 and HIF-1α expressions. *Results:* The 20-month survival of HER2(-) and HIF-1α(-) cases (n = 6) was 100% and that of HER2(+) and HIF-1α(+) cases (n = 4) was 37.5% ($p = 0.0032$). *Conclusions:* In this first prospective analysis of patients with uterine cervical carcinoma treated with concurrent chemoradiotherapy, concomitant expression of HER2 and HIF-1α was suggested to be a strong indicator of poor prognosis. A novel therapy including molecular targeted therapy such as anti-HER2 and anti-HIF-1α may be worth considering in patients with concomitant expression of HER2 and HIF-1α.

Key words: Uterine cervical carcinoma; Chemoradiation; HER2, HIF-1α.

Introduction

HER2 is an epidermal growth factor receptor (EGFR)-like protein [1] considered to be related with tumor growth [2]. However, no ligands that bind directly to HER2 have been detected. On the other hand, in clinical practice, HER2 has been widely recognized to be a very significant poor prognostic factor of breast cancer [3, 4].

Moreover, the anti-HER2 molecular targeted drug trastuzumab has already been produced and used worldwide to counter the overexpression of HER2 in breast cancer, with successful results [5-7]. Furthermore, retrospective studies have reported that expression of HER2 might be correlated with poor prognosis in uterine cervical carcinoma [8-10].

Hypoxia-inducible factor 1 (HIF-1) is composed of the heterodimers HIF-1α and HIF-1β, which are bHLH/PAS proteins [11]. HIF-1α is an indicator of hypoxia in response to the cellular O_2 concentration and is rapidly ubiquitinated and degenerated under aerobic conditions [11-13]. Recently, HIF-1α expression has been correlated with hypoxia in uterine cervical carcinoma [14, 15]. Moreover, a retrospective study has reported that the expression of HIF-1α in uterine cervical carcinoma is correlated with poor prognosis [16].

Activity of tumor proliferation and hypoxia are very important radioresistant factors. However, to date, no prospective studies have been performed to evaluate the radiation sensitivity and treatment outcomes of patients with uterine cervical carcinoma relative to HER2 and HIF-1α expressions. We are engaged in a prospective phase II study of concurrent chemoradiotherapy for locally advanced uterine cervical carcinoma using nedaplatin, a new platinum agent; this study has been designated as KGROG0501 (Kitasato Gynecologic Radi-

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Fig. 1a

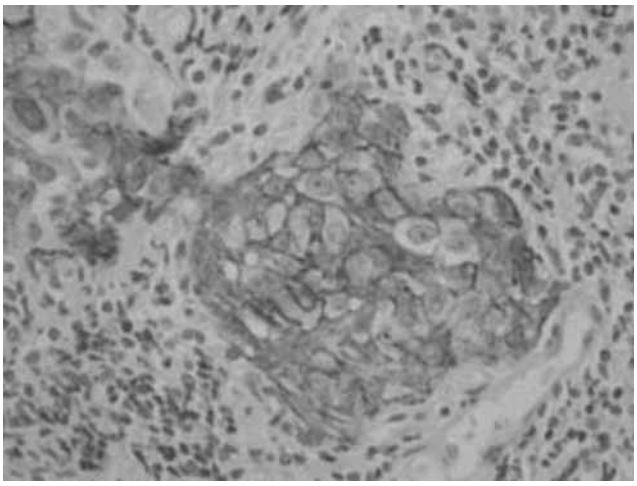

Fig. 1b

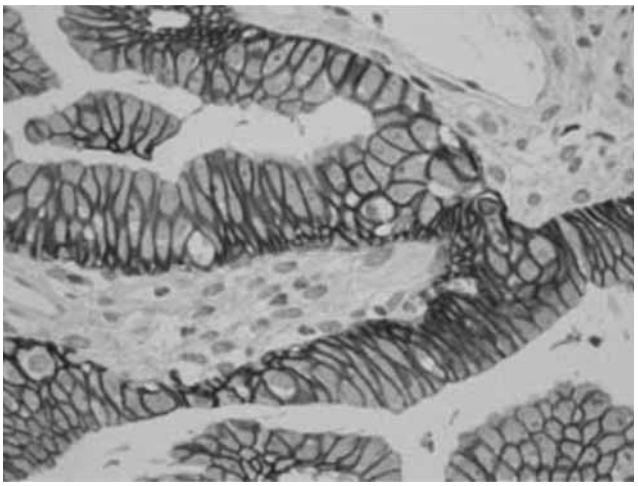

Fig. 2a

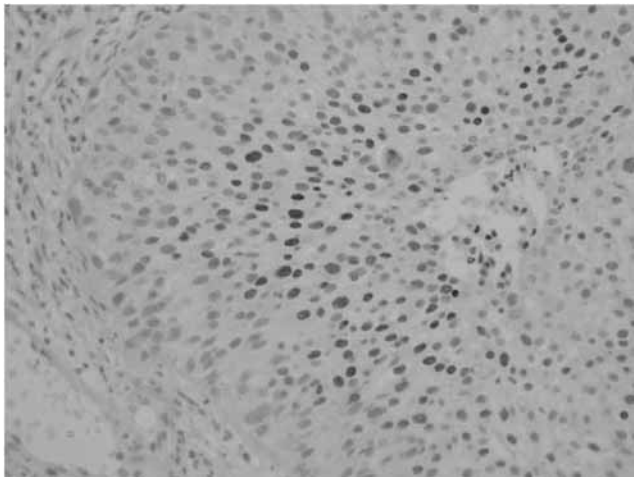

Fig. 2b

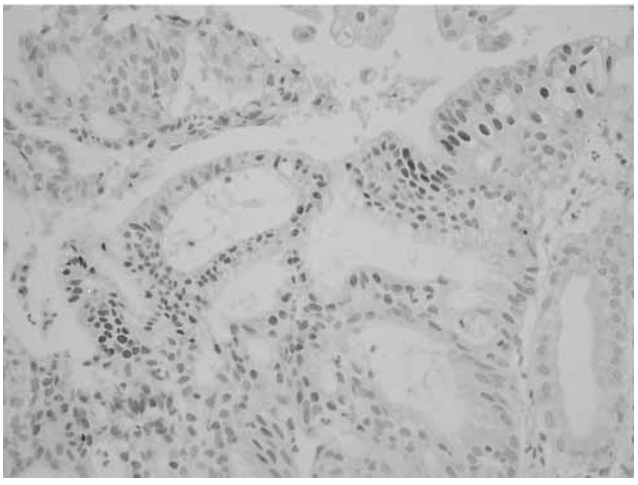

Figure 1. — Expression of HER2 in squamous cell carcinoma (a) and in adenocarcinoma (b) of the uterine cervix using immunohistochemistry (× 200).
Figure 2. — Expression of HIF-1α in squamous cell carcinoma (a) and in adenocarcinoma (b) of the uterine cervix using immunohistochemistry (× 200).

ation Oncology Group Study 0501) and its protocol has been reported elsewhere [17, 18]. In March 2009, we performed an interim analysis of the KGROG0501results and the correlation between HER2 expression, HIF-1α expression, and treatment outcomes was analyzed prospectively.

Patients and Methods

Between June 2005 and April 2008, 25 patients with locally advanced uterine cervical carcinoma were enrolled in KGROG0501. Patient characteristics are listed in Table 1. The median age was 55.5 years (range, 31-75 years). The clinical stages based on the International Federation of Gyenecology and Obstetrics (FIGO, 1994) were Ib2/IIb/ IIIb/IVa in 1/2/21/1 cases, respectively. Nineteen cases had squamous cell carcinoma and six had adenocarcinoma. The protocol therapy of KGROG0501 was completed in all patients.

HER2 expression was analyzed using an immunohistochemical kit for HER2 (Hercept Test II kit, DAKO, Denmark) on

Table 1. — *Patient characteristics*.

Age	
31-75 years (median: 55.5 years)	
*PS**	
0	5
1	20
2	0
Clinical Stage	
Ib2	1
IIb	2
IIIb	22
IVa	1
Histopathology	
Squamous cell carcinoma	19
Adenocarcinoma	6
MTD**: 30-100 mm (median: 65.0 mm)	

*MTD: maximal tumor diameter.
**Performance Status.

Fig. 3

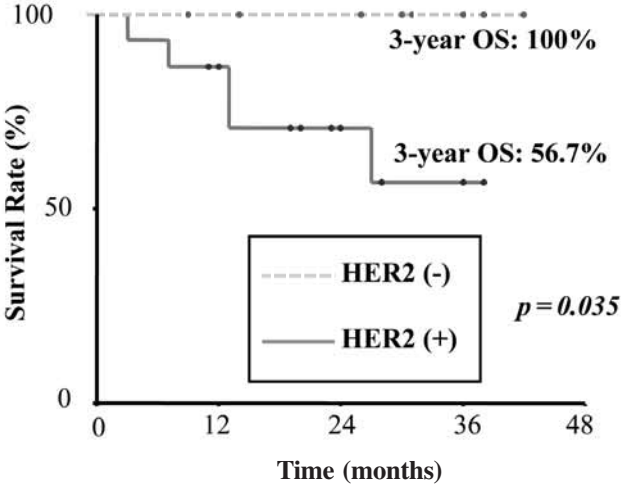

Fig. 4

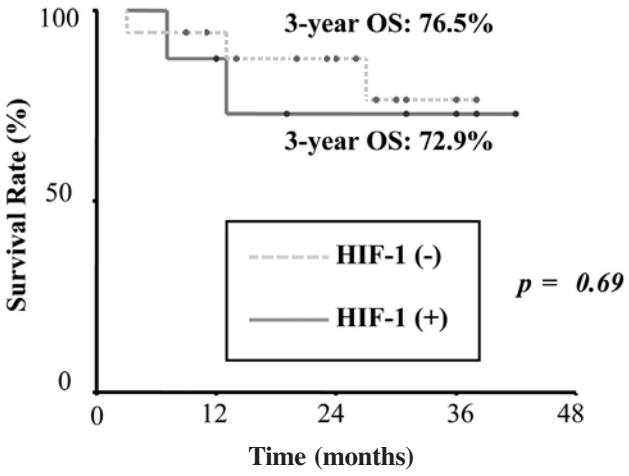

Fig. 5

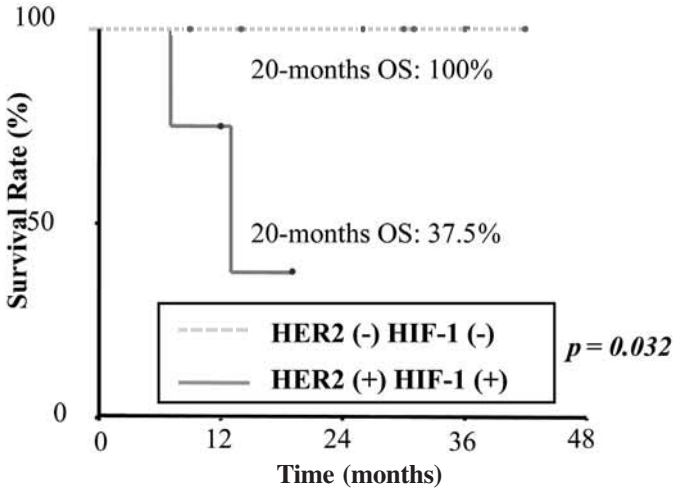

Figure 3. — Overall survival curves with and without HER2 expression.

Figure 4. — Overall survival curves with and without HIF-1α expression.

Figure 5. — Overall survival curves with and without concomitant expressions of HER2 and HIF-1α.

biopsied sections according to the manufacturer's instructions. HIF-1α expression was analyzed using immunohistochemical method (LSAB2 kit, DAKO, Denmark). Briefly, 5-μm thick sections were deparaffinized in xylene and rehydrated. They were treated with 0.3% hydrogen peroxide for 5 min. Then, the sections were incubated in 10 mM citrate buffer at 121°C for 15 min. After blocking with 10% normal swin serum (DAKO), the sections were incubated with anti HIF-1α mouse monoclonal antiboby (BD Biosciences, USA, 1:1000 dilution) overnight at room temperature. As negative controls, normal mouse or rabbit serum at the same dilution was used. Detection was achieved with diaminobenzidine reaction for 10 min. Nuclei were lightly counterstained by Mayer's hematoxylin.

Level of HER2 expression was ranked to 0 to 3+ according to the United States Food and Drug Administration (FDA) guidelines. For statistical analysis, 0 was assigned to HER2 (-) and 1+ to 3+ to HER2 (+).

Nuclear labeling indices of HIF-1α were calculated for all specimens. The cut-off value of HIF-1 (+) was set at ≥ 35% nuclear positivity. For statistical analysis, a survival curve was constructed by the Kaplan-Meier method. The log-rank test was performed to evaluate statistical significance. A p-value < 0.05 was defined as statistically significant. Comparisons among patients with and without HER2 and HIF-1α expressions and survival rates were performed.

Results

HER2 was expressed as 0 in ten cases, 1+ in nine cases, 2+ in five cases, and 3+ in one case. Figure 1a indicates the positive expression of HER2 in squamous cell carcinoma of the uterine cervix. Figure 1b indicates the positive expression of HER2 in adenocarcinoma. Only eight patients had positive expression of HIF-1α. Figure 2a and 2b indicate the positive expression of HIF-1α in squamous cell carcinoma and adenocarcinoma, respectively.

The 3-year overall survival of HER2-positive patients was 100% and the 3-year overall survival of HER2-negative patients was 56.7% (Figure 3). These differences were statistically significant (p = 0.035). The 3-year overall survival of HIF-1α-positive patients was 76.5% and the 3-year overall survival of HIF-1α-negative patients was 72.9% (Figure 4). These were almost the same (p = 0.69). However, in an analysis of the combination of HER2 and HIF-1α, the 20-month overall survival of HER2-negative and HIF-1α-negative patients was 100% and the 20-month overall survival of HER2-positive and HIF-1α-positive patients was 37.5% (Figure 5). This difference was statistically significant (p = 0.032) and the difference in the survival curves was quite large.

Discussion

Concurrent chemoradiotherapy is recognized to be standard treatment for locally advanced uterine cervical carcinoma. However, the 5-year overall survival of these patients was no more than 73% [19]. This indicated that he same disease status leads to different results. About 70% of the patients with locally advanced uterine cervical carcinoma who received concurrent chemoradiotherapy achieved complete recovery and about 30% of them failed to death. To date, many studies have been performed to overcome these problems from the era of radiation therapy alone as the standard therapy for locally advanced uterine cervical carcinoma.

Oka *et al*. investigated the pretreatment expressions of p27 and p53 in 77 patients with squamous cell carcinoma of the uterine cervix treated with radiation therapy alone and concluded that high expression of p27 and low expression of p53 were correlated with poor overall survival [20]. Manganese superoxide dismutase (Mn-SOD), which removes radiation-induced toxic superoxide radicals, was also found to be a prognostic factor for patients with uterine cervical carcinoma treated with radiation therapy alone [21]. The 5-year overall survival of Mn-SOD-positive patients was 42.5%, significantly poorer than the 77.0% of Mn-SOD-negative patients ($p < 0.05$). Other cell cycle- or tumor proliferation-related proteins were investigated such as p63 [22], vascular endothelial growth factor (VEGR) [23], cyclooxygenase-2 expression (COX-2) [24, 25], and survivin [26]. All of these factors were correlated with clinical outcomes of radiation therapy alone. In addition to cell-cycle- or tumor-proliferation-related proteins, the genotype of human papillomavirus was investigated [27]. However, there was no evident correlation between overall survival and the genotype of human papillomavirus. On the other hand, imaging approaches to predict the clinical outcomes have been performed. Kodaira *et al*. reported that magnetic resonance imaging indicated the prognosis of Stage III uterine cervical carcinoma to predict distant metastasis [28]. Kidd *et al*. reported the usefulness of positron emission tomography to evaluate the pretreatment maximum standardized glucose uptake values of the cervical tumor, which was correlated with prognosis [29]. However, no prospective studies have been performed in conjunction with prospective clinical trials.

HER2 has been investigated previously. Nakano *et al*. reported that the 5-year overall survival rate of HER2-positive patients with locally advanced uterine cervical carcinoma treated with radiation therapy alone was 45.1% and that of HER2-negative patients was 75.6% [30]. This was statistically significant ($p < 0.01$). Furthermore, Niibe *et al*. investigated patients with advanced uterine cervical carcinoma with paraaortic lymph node metastasis treated with radiation therapy and concluded that the prognosis of HER2-positive patients is poorer than that of HER2-negative patients [9]. Recently, Yamashita *et al*. reported that the prognosis of HER2-positive patients with locally advanced uterine cervical carcinoma treated with chemoradiation is poorer, although the difference was only marginally statistically significant [10]. All of these findings suggested that the prognosis of HER2-positive patients with uterine cervical carcinoma treated with radiation therapy is poorer than that of HER2-negative patients. However, these studies were all retrospective analyses. No prospective studies concerning HER2 in cases of locally advanced uterine cervical carcinoma have been performed yet. The current study is the first prospective clinical study to evaluate the role of HER2 in locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy.

The current study indicated that the 3-year overall survival of HER2-positive patients was 100% and the 3-year overall survival of HER2-negative patients was 56.7%. These differences were statistically significant ($p = 0.035$). This result is the same as in the previous retrospective studies. Moreover, this is first confirmation prospectively that HER2 is the prognostic factor of uterine cervical carcinoma.

HIF-1α has also been investigated previously. Dellas *et al*. reported that the 5-year overall survival of HIF-1α-positive patients with locally advanced uterine cervical carcinoma was 45% and that of HIF-1α-negative patients was 92% ($p < 0.02$) [31]. Ishikawa *et al*. reported that expression of HIF-1α predicts frequent distant metastases ($p = 0.03$) [16]. Other studies of almost the same results have been reported already [32, 33]. However, the current study indicated that the 3-year overall survival of HIF-1α-positive patients was 76.5% and the 3-year overall survival of HIF-1α-negative patients was 72.9%. These survival rates were nearly the same ($p = 0.69$) and contrasted with the results of previous studies. Nonetheless, the interpretation of these prospective results must be made very cautiously.

The results of this prospective study are interim ones, not final results. The follow-up period was not especially long. The mechanism of the correlation between HIF-1α and the poor clinical prognosis of radiation therapy is that HIF-1α is strongly correlated with hypoxia and that hypoxic tumor cells are resistant to irradiation, so tumor cells left after radiation therapy lead to a poor prognosis [14, 15]. However, if radiation-resistant hypoxic cells are dormant cells, hypoxic cells have no strong proliferation potential, which does not result in imminent patient death but in patient survival for several more years. The results of the current study could indicate this situation. The evidence to support this hypothesis is that in the analysis of the combination of HER2 and HIF-1α expressions, the 20-month overall survival of HER2-negative and HIF-1α-negative patients was 100% and the 20-month overall survival of HER2-positive and HIF-1α-positive patients was 37.5%, which was statistically significant ($p = 0.032$) and the difference in the survival curves was quite large. Patients with HIF-1α-positive expression coincident with HER2-positive expression, indicative of a highly proliferative state of tumor cells, were predicted to have very bad results. On the other hand, patients with HIF1α-negative expression coincident with HER2-nega-

tive expression, achieved a 20-month survival of 100%. This is the first investigation of this combined expression of HER2 and HIF-1α, which is a clear and reasonable result. Further investigation is required to validate this combination pretreatment test as a promising predictor of the prognosis of locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy. Furthermore, applying molecular targeted therapy of both anti-HER2 and anti-HIF-1α will warrant a better prognosis of locally uterine cervical carcinoma treated with concurrent chemoradiotherapy.

In conclusion, this is the first prospective analysis to evaluate the prognostic role of the expressions of HIF-1α and HER2 in uterine cervical carcinoma treated with concurrent chemoradiotherapy. The concomitant expression of HER2 and HIF-1α in patients with locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy was a strong predictor of poor prognosis. A novel therapy including molecular targeted therapy may be worth considering in patients with concomitant expression of HER2 and HIF-1α.

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