Detection of Epidermal Growth Factor Receptor in the Serum of Patients with Cervical Carcinoma

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ABSTRACT

Epidermal growth factor receptor (EGFR) is overexpressed in a variety of malignancies, including breast, lung, gastric, and cervical carcinoma. Its overexpression has been associated with disease progression or poor prognosis in patients with cervical carcinoma. In the present study, the levels of EGFR were determined in serum from 38 patients with cervical carcinoma [invasive or recurrent carcinoma (n = 26) and carcinoma in situ (CIS; n = 12)] and 38 healthy female controls using ELISA. The mean serum level for EGFR in patients with invasive or recurrent carcinoma (165 ± 60 fmol/ml) was significantly elevated (P < 0.0001) compared with that of healthy controls (66 ± 17 fmol/ml) and also higher (P = 0.015) than that of patients with CIS (126 ± 25 fmol/ml). In addition, there was a significant difference in the mean serum levels of EGFR between patients with CIS and healthy controls (P < 0.0001). Thirty-five patients (92%) with cervical carcinoma [invasive or recurrent (n = 24) and CIS (n = 11)] had elevated serum EGFR levels above the cutoff value of 100 fmol/ml (defined as 2 SD above the mean of the controls). In conclusion, the serum EGFR level was elevated in a significant proportion of patients with cervical carcinoma, and it demonstrated an increasing tendency according to disease progression from normal tissue through CIS to invasive cervical carcinoma. Therefore, it may have a potential usefulness as a biological marker of cervical carcinoma.

INTRODUCTION

EGFR is a Mr 170,000 glycoprotein composed of three domains: an internal domain with tyrosine kinase activity, an external ligand-binding domain (extracellular domain), and a transmembrane domain (1–4). The binding of EGF or transforming growth factor α to EGFR activates a signal transduction pathway and results in cell proliferation (1–4). In addition, EGFR has been known to play a role in cell differentiation, enhancement of cell motility, protein secretion, neovascularization, invasion, metastasis, and resistance of cancer cells to chemotherapeutic agents and radiation (3, 4).

The overexpression of EGFR has been reported in various types of malignancies (5–8). Moreover, EGFR overexpression is associated with poor prognosis or advanced stage in breast, bladder, head and neck, esophageal, lung, and gastric carcinoma (5–8). In cervical carcinoma, overexpression of EGFR in tumor tissue has been reported in 6–85% of cases among different studies (9–18). Several studies have suggested that overexpression of EGFR correlates with more aggressive biological behavior, including large tumor size, lymph node metastasis, and poor prognosis (9, 12, 13, 15, 17). Furthermore, dysregulation of EGFR may play an important role in the progression of cervical carcinoma from normal epithelium through various stages of cervical intraepithelial neoplasia to invasive cervical carcinoma (18–22).

The most common method used to detect overexpression of EGFR is immunohistochemical staining on paraffin-embedded specimens (7, 8, 10–13, 15, 16, 18). Recently, ELISA has been developed and allows quantitative determination of the ECD of EGFR in the serum of cancer patients (8, 23). A significantly elevated serum level of EGFR in cancer patients compared with healthy controls was demonstrated in lung and gastric cancer (8, 23). Determination of serum levels of EGFR in cervical carcinoma patients may provide valuable information, considering the high expression of EGFR in the cervical carcinoma tissue as well as the potential usefulness of serum assay in the clinical setting (8, 10, 12, 13, 17, 18). However, to our knowledge, there has been no report of the serum EGFR in cervical carcinoma. In this study, we determined the levels of EGFR ECD in the serum of patients with cervical carcinoma, which is an important health problem in the world (24). The association between the levels of EGFR in the serum and clinicopathological characteristics was investigated.

MATERIALS AND METHODS

Patients. Serum levels of EGFR ECD were measured in 38 patients with pathologically confirmed cervical carcinoma.

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The abbreviations used are: EGFR, epidermal growth factor receptor; ECD, extracellular domain; CIS, carcinoma in situ.
between January and August 1996 at Korea University Medical Center in Seoul, Korea (Republic). Only patients who were recently diagnosed but untreated (34 patients) or recurred after treatment (4 patients) were included in this study. Blood samples were collected before treatment. Hospital records and pathology slides of the patients were reviewed. Stage grouping was made according to Fédération Internationale des Gynécologues et Obstétristes classification (1994; Ref. 25).

**Determination of Serum EGFR Level.** After 6 h of fasting, venous blood samples were collected with Vacutainer glass tube without additive (Becton Dickinson, Franklin Lakes, NJ). The serum was then separated by centrifugation at 3000 rpm for 10 min and stored at −70°C until the time of analysis. Serum samples were also obtained from 38 healthy female control individuals.

Serum samples from the patients and controls were assayed for the levels of EGFR ECD by a sandwich ELISA, which uses a mouse monoclonal capture antibody against EGFR precoated onto a microtiter plate and a biotinylated detector antibody against EGFR, according to the recommendation of the manufacturer with human EGFR quantitative ELISA assay kit (Calbiochem, Cambridge, MA).

**Statistical Analysis.** Mean and SDs in terms of fmol EGFR ECD/ml were calculated for the samples from healthy controls and cervical carcinoma patients. The Mann-Whitney U test was used to compare different groups for continuous variables, including the serum levels of EGFR. We defined a positive elevation of serum EGFR level as any value >2 SDs above the mean value of healthy controls.

**RESULTS**

**Patient Characteristics.** The median age of the patients with cervical carcinoma was 51 years (range, 22–72), whereas that of the control group was 49 years (range, 23–69). Twelve patients had CIS, 22 patients had invasive carcinoma, and 4 patients had recurrent disease after treatment. Among the patients with CIS, 10 had total abdominal hysterectomy and 2 received laser conization as the primary treatment. All patients with stage I or IIA underwent radical hysterectomy, whereas those with stage IIB disease had radiotherapy as the primary treatment. In recurrent cases, systemic chemotherapy or radiotherapy was used.

In terms of initial stage for four patients with recurrent disease, two had IB, one had IIB, and initial stage was not available in one patient. As a prior treatment, radiotherapy was performed in two patients, and two patients received surgical resection and chemotherapy with or without radiotherapy. Two patients had local recurrence, whereas two had regional recurrence (paraarotic lymph nodes). Table 1 lists additional patient characteristics.

**Serum Levels of EGFR.** In the control group, the mean serum level of EGFR was 66 ± 17 fmol/ml (median, 68; range, 29–101). There was no statistically significant difference in the serum levels of EGFR between premenopausal and postmenopausal women in the control group (P = 0.121). The mean serum level of EGFR in the invasive or recurrent cervical carcinoma patients was 165 ± 60 fmol/ml (median, 164; range, 76–384), whereas in CIS patients was 126 ± 25 fmol/ml (median, 120; range, 76–176). When compared with the control group, both groups demonstrated a significantly elevated level of EGFR (P < 0.0001 in both groups). A statistically significant difference in the mean serum EGFR levels was also observed between the invasive or recurrent carcinoma group and the CIS group (P = 0.015). There was no significant difference in the mean serum levels of EGFR between patients with invasive carcinoma and those with recurrent disease (154 ± 42 fmol/ml; range, 76–256 fmol/ml versus 227 ± 108 fmol/ml; range, 147–384 fmol/ml; P = 0.110). However, the mean serum level of EGFR in patients with invasive carcinoma excluding recurrent cases was significantly higher compared with that of CIS patients (P = 0.041; Fig. 1).

Age and menopausal status did not correlate with serum EGFR levels. In patients with invasive carcinoma, serum EGFR levels were not associated with stage and primary tumor size (Table 2). The cutoff value for EGFR positivity was 100 fmol/ml, using the definition of positive serum elevation of EGFR any value >2 SDs above the mean of the control group. On the basis of these criteria, positive elevation of serum EGFR was demonstrated in 35 patients with cervical carcinoma (92%). In patients with CIS, serum EGFR demonstrated positive elevation in 11 of 12 (92%), whereas in those with invasive or recurrent disease, elevated serum EGFR level was detected in 24 of 26 (92%; Fig. 1).

**DISCUSSION**

In cervical carcinoma, the overexpression of EGFR has been usually evaluated in tumor tissue with immunohistochemical staining or radioligand-binding assay (9–16, 18). The serum assay for oncoproteins with ELISA can be easily and repeatedly

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**Table 1**  Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
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<tr>
<td>Control group</td>
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<tr>
<td>Age</td>
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<tr>
<td>Median (years)</td>
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<tr>
<td>Premenopause</td>
<td>20 (53)</td>
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<td>Postmenopause</td>
<td>18 (47)</td>
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<tr>
<td>Patient group</td>
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<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
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<td>22–72</td>
</tr>
<tr>
<td>Median (years)</td>
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</tr>
<tr>
<td>Premenopause</td>
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<td>Postmenopause</td>
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<td>Surgical resection</td>
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<td>Yes</td>
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<tr>
<td>No</td>
<td>12 (32)</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
<td>IB</td>
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<tr>
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<tr>
<td>IIB</td>
<td>7 (18)</td>
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<tr>
<td>IV</td>
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<tr>
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<td>37 (97)</td>
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<tr>
<td>Adenosquamous</td>
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performed because of its minimal invasiveness compared with assays using surgically obtained tissue materials (8, 26).

In the current study, the mean serum level for the EGFR in the cervical carcinoma patients was significantly elevated compared with that of healthy controls, with 92% of patients demonstrating a positive elevation of EGFR. Previously, the overexpression of EGFR in the tissue specimen has been reported in 6–85% of cases (9–18). It is difficult to directly compare the positivity of serum EGFR with its overexpression in tumor tissue because of different methods used in measuring the serum and tissue EGFR levels. In addition, some degree of expression of EGFR has been reported in normal cervical tissue (10, 12, 13, 15, 17, 19, 20, 22). Therefore, it would be desirable to compare the expression of EGFR in matched samples of serum and tissue in a larger sample size.

Although the serum EGFR levels were significantly elevated in the cervical carcinoma patients compared with controls, EGFR was also detected in the serum of all control women in this study. EGFR is present on all epithelial and stromal cells as well as selected smooth muscle cells, and a detectable level of EGFR in the serum of healthy control individuals has been reported in previous studies (3, 8, 23). Therefore, a certain amount of EGFR ECD released from normal tissue may explain the baseline level of EGFR in the serum of controls. Accordingly, the significantly increased level of serum EGFR in cervical carcinoma patients is most likely resulted from the overexpression of EGFR in the tumor tissue. However, the exact mechanism of increased EGFR in the serum of cervical carcinoma patients remains to be answered, at this point.

Several studies reported higher expression of EGFR in cervical intraepithelial neoplasia lesions including CIS when compared with the surrounding normal tissue. On the basis of these observations, EGFR has been suggested as a potential marker of progression in cervical precancer lesions (18–22). Moreover, the important role of EGFR in the carcinogenesis of cervical carcinoma has been suggested by a few studies that showed that human papillomavirus-associated gene products such as E6 protein up-regulate EGFR expression (27, 28). In the present study, the serum levels of EGFR were significantly elevated in CIS patients when compared with those of controls. This result suggests that overexpression of EGFR in cervical carcinoma tissue may lead to the elevated levels of EGFR in the serum even at the early stage as CIS. The mechanism of elevated serum EGFR levels in patients with CIS, in which tumor cells do not penetrate the basement membrane of cervical epithelium, remains to be defined, at this point.

Several studies using tumor tissue demonstrated that overexpression of EGFR might be associated with pathological features of more aggressive disease such as a large tumor size and lymph node metastasis in cervical carcinoma (9, 12, 13, 17). In the present study, the mean serum level of EGFR was significantly elevated in patients with invasive or recurrent cervical carcinoma when compared with that of CIS patients. Such increasing tendency of serum EGFR level according to disease progression from normal tissue through CIS to invasive cervical carcinoma may suggest the association of the elevated level of EGFR with aggressive behavior of cervical carcinoma. However, the serum EGFR levels did not correlate with primary tumor size and stage in patients with invasive carcinoma, and a limited number of patients in the current study may explain these findings.

The current results also suggest the possible role of serum EGFR as a biological marker of cervical carcinoma. The role of EGFR as an independent predictor of poor prognosis in cervical carcinoma has been suggested in several studies using tumor tissue, although there are a few reports questioning its role as a prognosticator (9, 12–15, 29). Serum EGFR may also be useful in monitoring cervical carcinoma patients after surgery or radiotherapy as suggested in serum c-erbB-2, which is highly homologous to EGFR, in lung and breast cancer (26, 30). Therefore, there is a possibility that the determination of serum EGFR level is useful for the prediction of prognosis or follow-up after treatment, considering the simplicity and objectivity of the method. However, prospective studies with large enough sample size are required to confirm these findings.
cohorts including serial follow-up of serum EGFR levels and concurrent analysis for its expression in tumor tissue would be necessary to answer these speculations.

In conclusion, the serum EGFR level was elevated in a significant proportion of patients with cervical carcinoma, and it demonstrated an increasing tendency according to disease progression from normal tissue through CIS to invasive cervical carcinoma. Serum EGFR may be a potential biological marker of cervical carcinoma, although additional studies with a large number of patients are required to establish its clinical usefulness.

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