

# Correlation between EGFR and c-erbB-2 Oncoprotein Status and Response to Neoadjuvant Chemotherapy in Cervical Carcinoma

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## Abstract

Neoadjuvant chemotherapy prior to definitive radical surgery or radiotherapy may be effective in reducing tumor volume or clinical stage and may even enhance pelvic control and survival. However, there are significant limitations to the use of neoadjuvant therapy in the non-responder group. They include delayed total treatment course, the presence of drug resistant clones which result in accelerated tumor growth, and limited bone marrow reserve for subsequent definitive therapy. Thus, there is a need to identify parameters providing a more precise indication of the response to neoadjuvant chemotherapy in patients with invasive cervical cancer. From Jan. 1995 to Jan. 1996, neoadjuvant chemotherapy with 3 courses of cisplatin and vincristine was used in 32 patients with invasive cervical cancer (FIGO stage Ib to IIIb; tumor size greater than 2 cm). Prior to chemotherapy, quantitative tissue levels of epidermal growth factor receptor (EGFR) and c-erbB-2 oncogene protein were measured by using an enzyme-linked immunosorbent assay (ELISA). Tumor size was estimated before and after chemotherapy. Relations between oncoproteins and reductions of tumor size were evaluated. Tumor size prior to neoadjuvant chemotherapy did not show any correlation with either the concentrations of EGFR or c-erbB-2 oncoprotein. As well, the tumor reduction index did not manifest any correlation with EGFR, it did had an inverse linear correlation with the c-erbB-2 oncoprotein levels ( $R_s = -0.71$ ,  $P < 0.05$ ). The results of this study suggest that c-erbB-2 oncoprotein is associated with a reduced response to neoadjuvant chemotherapy in primary treatment of invasive cervical cancer and may be useful in directing therapeutic approaches.

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**Key Words:** EGFR, c-erbB-2, neoadjuvant chemotherapy, cervical cancer

## INTRODUCTION

Cancer of the uterine cervix is the most common malignancy in Korean women. The incidence rate of cervical cancer in Korea is low before 25 years of age, but it increases after age 35, reaching a peak in the 60th year. It accounts for 29.9 cases per 100,000 population.<sup>1</sup> Although early-stage cervical carcinoma can be treated successfully with definitive surgery or radiation therapy, carcinoma of the cervix has been demonstrated to have a high treatment failure rate and poor survival in association with risk factors including advanced stage,<sup>2</sup> regional lymph node me-

tastasis<sup>3,4</sup> and bulky tumor.<sup>4,5</sup>

The use of neoadjuvant chemotherapy in cervical cancer prior to definitive local therapy seems to be of therapeutic interest. There have been considerable clinical trials using neoadjuvant chemotherapy in cervical cancer over the past 20 years. Several phase II trials have obtained encouraging results using induction chemotherapy to treat locally advanced cervical carcinoma.<sup>6-9</sup> Recently, 6 randomized trials of various regimens of neoadjuvant chemotherapy for patients with carcinoma of the cervix have been reported.<sup>5,10-14</sup> However, except for the results of Sardi et al. none of these results has shown any advantages for pelvic control or survival. These findings prompted us to investigate the parameters providing a more precise indication of the response to neoadjuvant chemotherapy for this disease.

It has been established over recent decades that the molecular mechanisms implicated in the development and uncontrolled proliferation of cancers involve

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abnormalities of oncogenes and growth factor-receptor systems. As our knowledge of the role of regulatory genes in oncogenesis increases, the quantitation of their expressed products will undoubtedly find increased clinical utility. Amplification of certain oncogenes or an increase in their oncoproteins have been shown to have prognostic significance in several cancers.<sup>15-18</sup>

c-erbB-2 oncoprotein is a 185-KDa membrane-bound glycoprotein. It is a receptor on the cytoplasmic membrane that is homologous to the epidermal growth factor receptor (c-erbB-1). The c-erbB-2 oncogene was independently discovered by several groups and consequently is referred to by various names, including *HER-2*<sup>19</sup> and *neu*.<sup>20,21</sup> Overexpression of c-erbB2 has been demonstrated in 4% to 38% of patients with cervical cancer and has been found by most, but not all, investigators to be associated with poor prognosis.<sup>22-24</sup> There have been a few studies suggesting that overexpression of growth factor receptors may be associated with resistance to chemotherapy in cancer patients.<sup>25,26</sup> Allred et al. showed that for some unknown reasons breast cancer patients with high c-erbB-2 levels showed resistance to adjuvant chemotherapy.<sup>25</sup> In addition, Tsai et al. have recently reported that increased resistance to the cytotoxicity of doxorubicin and cisplatin was directly related to increased c-erbB-2 expression in non-small cell lung cancer cell lines.<sup>27</sup>

In this study, we aimed to assess the relation of EGFR and c-erbB-2 oncoprotein expression in response to neoadjuvant chemotherapy in patients with cervical carcinoma.

## MATERIALS AND METHODS

### Patient entry criteria

Between January 1995 and January 1996, we enrolled 32 patients with histologically-invasive carcinoma of the uterine cervix. Eligibility criteria consisted of age less than 70 years with no other medical problem, a performance status greater than 70% in the Karnofsky scale, and a lesion size greater than 2 cm in maximum diameter measured by colposcopy and cervicography. Patients must not have received prior chemotherapy or radiotherapy. Other requirements were normal bone marrow reserve (hemoglobin

above 10 g/dL, leukocyte count more than 4,000/mm<sup>3</sup>, platelet count more than 100,000/mm<sup>3</sup>), renal function (blood urea nitrogen below 25 mg/dL, serum creatinine less than 1.5 mg/dL), liver function (bilirubin less than 1.5 mg/dL, transaminases less than 50 IU/L) and informed consent of the subjects. Staging evaluation included a complete medical history and physical examination, complete blood counts, biochemistries, chest x-ray, electrocardiogram, cystoscopy, intravenous pyelogram, and recto-sigmoidoscopy. Magnetic resonance imaging of the abdomen and pelvis was optional. Laboratory analyses and chest roentgenogram were repeated before each course of chemotherapy. The International Federation of Gynecology and Obstetrics staging criteria were used in this study.

### Measurement of EGFR and c-erbB-2 oncoprotein

Fresh cervical tissue samples were obtained from these patients before neoadjuvant chemotherapy and assayed as previously described.<sup>28</sup> Shortly after colposcopy-guided removal of cervical tissue, tissue specimens were stored at -70°C until processed. A portion of each tissue to be assayed for EGFR and c-erbB-2 oncoprotein status was examined to confirm the histological diagnosis of each cervical lesion.

For tissue preparation, after stripping blood and necrotic tissue, the obtained tissue samples were thawed on ice, placed in 10 volumes of ice-cold receptor buffer - 10 mM Tris HCL (pH 7.4), 1.5 mM EDTA, 10% glycerol, and 0.1% sodium azide and homogenized. The resulting mixture was centrifuged at 1,000-2,000 G for 10 minutes at room temperature, after which the supernatant was recovered. The total protein measured in the prepared supernatant was measured by Lowry et al.'s method.<sup>29</sup> The Oncogene enzyme immunoassay is a sandwich type immunoassay involving a mouse monoclonal capture antibody with rabbit antiserum as a detector. The microtiter assay plates coated with anti-EGFR monoclonal antibody and anti-c-erbB-2 monoclonal antibody were incubated with specimen (tissue supernatant) and the standard, respectively. During incubation, the receptor proteins present in the specimen or the standard were bound to the solid phase and the unbound materials present in the specimen were removed by fluid aspiration and washing. Antibodies conjugated with horseradish peroxidase were incubated

with the plates and when the receptor proteins were present in the specimen, the conjugates were bound to the receptors in the plate. Unbound conjugate was removed by aspiration, and the plates were washed. The plates were next incubated with enzyme substrate solution (hydrogen peroxide and O-phenylenediamine) to develop color which reflected the amount of bound EGFR and c-erbB-2 oncoprotein conjugates, respectively. The enzyme reactions were stopped by the addition of 2.5 N sulfuric acid and the intensity of the color developed was read using a spectrophotometer set at 490 nm. The intensity of the color formed was proportional to the concentration of receptors in the sample. A standard curve was obtained by plotting the standards concentration according to the absorbance, and the values of specimens were determined from the curve. The concentration of the EGFR and c-erbB-2 were calculated in fmole/mg cytosol protein by dividing the concentration of receptors by the concentration of cytosol protein. The cut-off points were 250 fmole/mg membrane protein for EGFR and 1,500 fmole/mg cytosol protein for c-erbB-2 oncoprotein.

### Treatment plan and outcome measures

All subjects were treated with the following chemotherapeutic schedule: cisplatin 50 mg/m<sup>2</sup> intravenously (IV) on day 1 with previous hydration; and vincristine 1 mg/m<sup>2</sup> IV on day 2. Treatment courses were repeated every 10 days for a total of three cycles.

All patients underwent colposcopy and cervico-

graphy to measure the cervical lesion size in centimeters at the time of staging and during the second and third admission for treatment. Three weeks after the third admission, the maximal tumor diameters were finally measured using colposcopy (Fig. 1). We used World Health Organization (WHO) guidelines for response criteria throughout the study.<sup>30</sup> We defined objective response as follows: complete response (CR) - complete resolution of all disease lasting at least 1 month; partial response (PR) - a decrease of greater than or equal to 50% in linear tumor measurement; stable disease (SD) - a decrease of less than 50% or an increase of less than 25% in the lesions; and progressive disease (PD) - 25% or more increase in the size of one or more lesions, or appearance of new lesions. The change in lesion size was used as a clinical marker for the assessment of treatment effect. Tumor reduction index (TRI) was defined as follows: TRI = (initial maximal tumor size - tumor maximal size after neoadjuvant chemotherapy) × 100/initial tumor size. Statistical analysis was carried out by chi-square 2×2 contingency tables with Yates' correction, Mann-Whitney U test, and Spearman rank correlation test. Differences were considered significant when the probability of error was below 5% (p<0.05).

## RESULTS

### Patient characteristics

Patient age ranged from 35 to 66 years with a median of 49.5 years. Regarding distribution according to stage, patients with stage II cervical carcinoma were the most numerous, accounting for 20 cases. According to cell type, 27 contained squamous cells and 3 were adenocarcinomas. When categorized by menstrual status, 17 cases of cervical carcinoma were premenopausal and 15 cases were postmenopausal. Positivity of EGFR and c-erbB-2 were found in 20 of 32 (62.5%) patients and in 17 of 32 (53.1%) cases, respectively (Table 1).

### Relation of response to oncoproteins status

Of 17 c-erbB-2 positive tumors, only 4 showed partial response, compared with 10 of 15 c-erbB-2 negative tumors (p<0.05). Of 20 EGFR positive

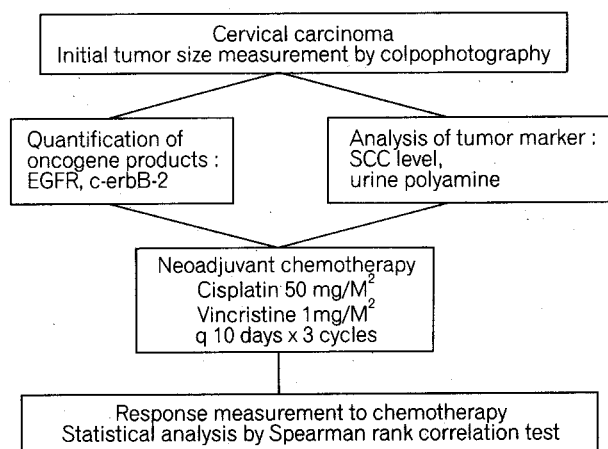


Fig. 1. Schematic diagram of study design.

tumors, 10 revealed a partial response, compared with 4 of 12 EGFR negative tumors (not significant) (Table 2). Levels of c-erbB-2 oncoprotein were significantly higher in the 18 non-responding patients than in 14 responding patients (median [range] 2870 [908–7055] vs 1248 [755–2165]) fmole/mg cytosol protein;  $p < 0.05$ ). There was no significant difference in EGFR levels between EGFR-positive responders and non-responders.

Table 1. Patient Characteristics at Study Entry

Characteristics	Number of cases (%)
Age (years)	
Median	49.5
Range	35–66
Menopausal status	
Premenopause	17 (53.1)
Postmenopause	15 (46.9)
Performance status*	
70–80%	2 (6.2)
90–100%	30 (93.8)
Clinical stage	
I	6 (18.8)
II	20 (62.5)
III	6 (18.8)
Histology	
Squamous carcinoma	27 (84.4)
Adenocarcinoma	3 (9.4)
Adenosquamous carcinoma	2 (6.2)
EGFR	
Positive	20 (62.5)
Negative	12 (37.5)
c-erbB-2 oncoprotein	
Positive	17 (53.1)
Negative	15 (46.9)

\*Karnofsky.

Table 2. Relation of Response to Oncoprotein Status

Response	EGFR (+) c-erbB-2 (-)	EGFR (-) c-erbB-2 (-)	EGFR (+) c-erbB-2 (+)	EGFR (-) c-erbB-2 (+)
Complete response	0	0	0	0
Partial response*	8	2	2	2
Stable disease	3	2	6	5
Progressive disease	0	0	1	1

\* $p < 0.05$ , c-erbB-2 (-) vs. c-erbB2 (+).

### Correlation between tumor size and oncoprotein status

The measured EGFR values and the initial tumor size showed a slight positive correlation ( $R_s = 0.39$ ) without any statistical significance (Fig. 2). We could

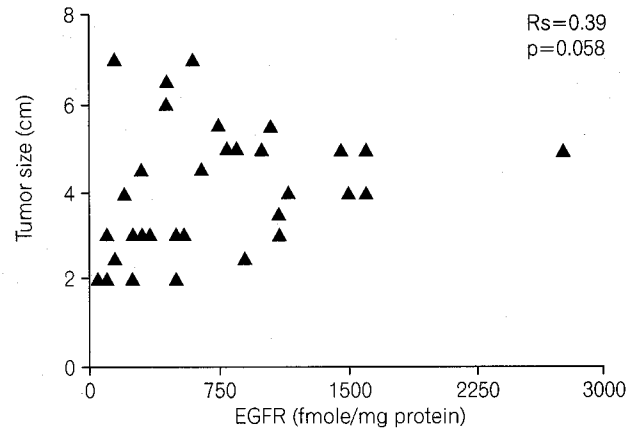


Fig. 2. Correlation between EGFR and tumor size.

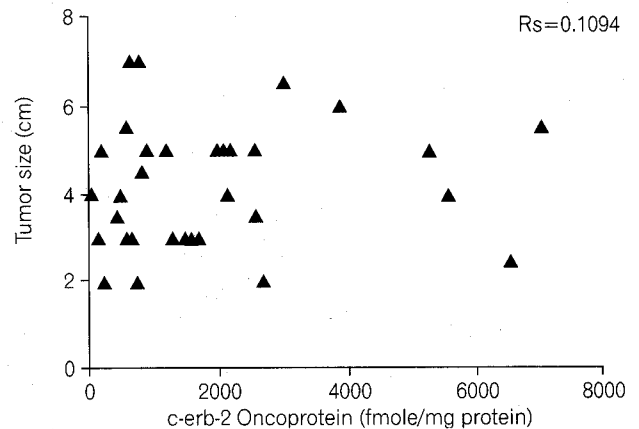


Fig. 3. Correlation between c-erbB-2 oncoprotein and tumor size.

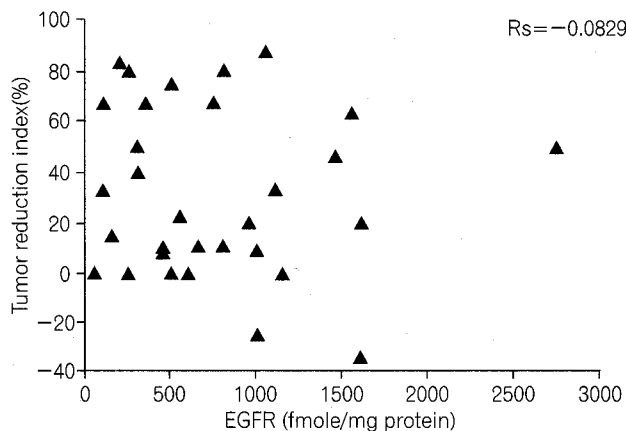


Fig. 4. Correlation between EGFR and tumor reduction index.

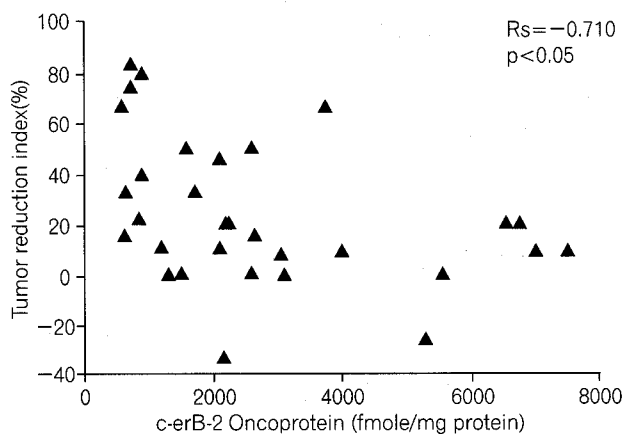


Fig. 5. Correlation between c-erbB-2 oncoprotein and tumor reduction index.

not demonstrate any positive correlation between c-erbB-2 oncoprotein and the initial tumor size (Fig. 3). No significant correlation was found between EGFR and the tumor reduction index ( $R_s = -0.08$ ) (Fig. 4). However, c-erbB-2 oncoproteins and the tumor reduction index did show an inverse linear correlation ( $R_s = -0.71$ ,  $p < 0.05$ ) (Fig. 5).

## DISCUSSION

Surgery and radiotherapy, the traditional cornerstones of management for patients with cervical cancer, have been considered to constitute the optimal therapeutic modalities. Much progress has been made in the early detection and control of cervical carcinomas since the introduction of the Papanicolaou

test. However, for patients with advanced stage, regional lymph node metastases and bulky tumor, the prognosis is poor even after surgery or radiation therapy.<sup>2-5</sup>

These unsatisfactory results have prompted investigators to introduce induction chemotherapy as an initial therapeutic approach for patients with cervical carcinoma.<sup>6-10</sup> The use of neoadjuvant chemotherapy is one of several studies being investigated to attempt to improve pelvic control and survival results. Although the initial use of chemotherapy in cervical cancer prior to definitive local therapy seems to be of therapeutic interest, significant theoretical limitations are associated with induction chemotherapy. A delay in local treatment due to drug-resistance may promote tumor growth, compromise subsequent surgery and/or radiotherapy, allow dissemination of micrometastases due to compromised host immunity, accelerate growth of drug-resistant cell lines, and cause treatment failure by direct toxic effect of chemotherapeutic agents. Thus, this led to the search for a new therapeutic approach to potentiate the neoadjuvant chemotherapy.

Epidermal growth factor receptor (EGFR) is a 170 kDa membrane-bound glycoprotein encoded by the c-erbB-1 oncogene. It was first purified from the A431 cell line which was derived from an epidermoid carcinoma of the vulva.<sup>31-34</sup> EGFR is a member of the growth factor receptor family of protein tyrosine kinases, a class of cell cycle regulatory molecules. The gene for the human EGFR is located on chromosome 7.<sup>35</sup> In the mid 1980s, the c-erbB-2 oncogene was revealed by several investigators. The *neu* oncogene was detected as a mutated transforming gene in neuroblastomas induced by ethylnitrosurea treatment of fetal rats.<sup>20,21</sup> The c-erbB-2 was a human gene discovered by its homology to the retroviral gene v-erbB.<sup>20</sup> *HER-2* was isolated by screening a human genomic DNA library for homology with v-erbB.<sup>19</sup> When the DNA sequences were subsequently determined, c-erbB-2, *HER-2*, and *neu* were found to represent the same gene. The c-erbB-2 oncogene is located on human chromosome 17q21 and encodes for c-erbB-2 m-RNA (4.6 kb), which translates c-erbB-2 oncoprotein (p185).<sup>46</sup> This oncoprotein is a glycoprotein which is a normal component of cytoplasmic membranes.

EGFR and c-erbB-2 have been reported as independent prognostic factors in various human tu-

mors.<sup>22,36-39</sup> In breast cancer cases, the overexpression of these oncogenes was reported to be related to the degree of response to chemotherapy.<sup>40-42</sup> Nicholson et al. reported that in recurrent breast cancer patients the response to adjuvant hormone therapy was poor when the initial EGFR level was high, and showed the possible use of the EGFR level as the prognostic index for predicting the therapeutic response.<sup>43</sup> However, Harris's work in 1990 showed no correlation between EGFR and the response rate to a single agent chemotherapy using mitoxantrone.<sup>41</sup> Our study also showed no correlation between EGFR and the tumor reduction index, suggesting that the sole measurement of EGFR may not be sufficient to predict the response to neoadjuvant chemotherapy.

There have been a few studies suggesting that overexpression of c-erbB-2 may be associated with resistance to chemotherapy in patients with breast cancer.<sup>25,26</sup> Gusterson studied 1,506 cases of breast cancer and reported that the overexpression of c-erbB-2 resulted in a poor response to chemotherapy.<sup>26</sup> Allred et al. showed that for some unknown reasons breast cancer patients with high c-erbB-2 levels showed resistance to adjuvant chemotherapy.<sup>25</sup> In addition, Tsai et al. have recently reported that increased resistance to the cytotoxicity of doxorubicin and cisplatin was directly related to increased c-erbB-2 expression in non-small cell lung-cancer cell lines.<sup>27</sup>

Our analysis of oncogene products in cervical cancer suggests that neoadjuvant chemotherapy may not result in a similar benefit for all patients with cervical carcinoma. It appears that patients with nonexistent or low c-erbB-2 expression may derive the greatest benefit from neoadjuvant chemotherapy. In contrast, no benefit from chemotherapy was observed in patients whose tumors overexpress c-erbB-2. This study showed an inverse proportional correlation between the level of c-erbB-2 oncoprotein and the tumor reduction index. That is, tumors with a high level of c-erbB-2 oncogene products showed no reduction of tumor size to three courses of neoadjuvant chemotherapy.

The results obtained in this study raise two possible explanations. The first is the possibility that the doses of chemotherapeutic agents used might have been too low to exert a significant antitumor effect in their own. Muss et al.<sup>44</sup> reported that overexpression of c-erbB-2 may be a useful marker to identify patients

who are most likely to benefit from high doses of adjuvant chemotherapy. The importance of drug dose for the response to cancer chemotherapy continues to be extensively studied.<sup>45</sup> The concept of dose intensity emphasizes the dose per unit time of drug delivery. For combination chemotherapy regimens, the clinical outcome depends per unit time, drug disposition, the resulting toxicity profile, and inherent tumor-cell sensitivity.

Another possible explanation for our findings is inadequate assessment of response. Several assessment techniques evaluating the change in tumor volume including pelvic examination,<sup>6,9</sup> ultrasonographic assessment,<sup>5</sup> magnetic resonance imaging technique,<sup>47,48</sup> and colpo-cervicography,<sup>12,13</sup> have been used to determine the response to chemotherapy in cervical cancer patients. However, a comparison of response rates in cervical cancer is difficult because noninvasive tumor measurements are less precise than surgical evaluations. Although recent reports suggest that magnetic resonance imaging techniques might become an important method in evaluating tumor volume even in the endophytic type of cervical cancer, it is also important to consider the financial costs in selecting the patients. Compared to such circumstances, cervicography is an objective, easy to perform, and precise method of assessment. Therefore, in this study, bidimensional tumor measurements from cervicography of every patient were obtained, but there were difficulties in cases of endophytic type cervical cancer.

In conclusion, this study suggests that c-erbB-2 oncoprotein is associated with a reduced response to neoadjuvant chemotherapy in the primary treatment of invasive cervical cancer and may be useful in directing therapeutic approaches.

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