**ERBB2 (HER2/neu) Oncogene Is Frequently Amplified in Squamous Cell Carcinoma of the Uterine Cervix**

A. B. Mitra, V. V. S. Murty, Mahendra Pratap, P. Sodhani, and R. S. K. Chaganti

Laboratory of Cancer Genetics and the Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York 10065, [A. B. M., V. V. V. S. M., R. S. K. C.], and the Institute of Cytology and Preventive Oncology, Indian Council of Medical Research, New Delhi, India [A. B. M., M. P., F. S.]

**Abstract**

We evaluated a panel of 22 protooncogenes for amplification in 50 primary, untreated squamous cell carcinomas of the uterine cervix. The tumors studied belonged to clinical stages II and III; histologically, the majority of them were moderately to well differentiated. Amplification represented by 5 or more copies was observed for the genes MYC/L1, SEA, CCND1, BCLI, and GLI in one case each (2%); HRAS in 2 cases (4%); and ERBB2 in 7 cases (14%). Amplification of ERBB2 ranged from 5 to 68 copies. In addition, 2 tumors with ERBB2 amplification showed additional restriction fragments suggesting possible mutation or rearrangement of the gene. The high incidence of ERBB2 amplification in cervical cancer suggests that this gene may play an important role in tumorigenesis.

**Introduction**

Carcinoma of the uterine cervix is the most common cancer which affects women in several parts of the world and causes high mortality. HPV16 and 18 have been implicated in the etiology of cervical cancer (1). HPV infection, although apparently necessary, is not considered to be sufficient for malignant transformation, suggesting a role for additional host cell genetic factors (1). These genetic factors involved in carcinogenesis of the uterine cervix are still not well understood. Specific chromosomal and molecular abnormalities including i(4p) or i(5p), rearrangements affecting chromosome 1 (2), amplification and overexpression of MYC (3–5), and allelic deletions affecting 3p (6), have been described.

Amplification of cellular protooncogenes has been associated with tumor progression (7), e.g.; MYCN in neuroblastoma (8), small cell lung cancer (9), and various other tumor types; and ERBB2 (HER2/neu) in breast (10), ovarian (11, 12), gastric (13, 14), and bladder carcinomas (15). In the present study we surveyed a panel of 22 protooncogenes and found frequent amplification of the ERBB2 gene in 14% of primary squamous cell carcinomas of uterine cervix.

**Materials and Methods**

The material studied comprised 50 consecutively ascertained primary, untreated tumor specimens obtained from patients with cancer of uterine cervix seen at the cancer clinic of Lok Nayak Jayaprakash Narayan Hospital, New Delhi, India. Clinically, the tumors studied belonged to stages II or III and histologically they were determined to be squamous cell carcinomas. Of the 50 tumors, 2 were poorly differentiated carcinomas, while the remaining were moderately to well differentiated carcinomas. The ages of the patients ranged from 30 to 80 years with a median of 45 years.

High molecular weight DNA was isolated from frozen tissues by proteinase K digestion, phenol-chloroform extraction and ethanol precipitation. Six μg of DNA were digested with the restriction enzymes EcoRI or HindIII, electro-photored on 0.8% agarose gels, and transferred onto nylon membranes. The same membranes were repeatedly hybridized, following stripping of probe, with [32P]dCTP-labeled probes. Posthybridization washes were performed at 65°C in 0.1% standard saline-citrate/0.5% sodium dodecyl sulfate for 40 min.

Hybridized membranes were exposed to Kodak XAR film and also read on a Betascope (Betagen) for quantitation of target gene signals in relation to control gene signals.

The details of 22 protooncogene probes used in this study and their map position are listed in Table 1. These probes were obtained either from the American Type Culture Collection or from the following investigators: MYC/L1 (F. Alt); MYCN (M. Schwab); MDRI (L. Roninson); MYC (W. Hayward); HRAS (E. H. Chang); SEA (P. N. Goodfellow); INT2 (G. Peters); CCND1 (A. Arnold); BCLI (C. M. Croce); KRAS2 (R. A. Weinberg); WNT1 (G. M. Schackelford); GLI (B. Vogelstein); ERBB2 (D. Slamon); and SRC (M. Bishop). An MD2 probe was derived by polymerase chain reaction from a complementary DNA sequence. Two polymorphic probes, D17S1 mapped to 17q and D12S2 mapped to 12p (both obtained from the American Type Culture Collection), and a probe for the immunoglobulin heavy chain gene joining region gene, JH (obtained from J. Ravetch), were used to control DNA loading. Gene copy number was determined as the ratio of target to control probe signals in tumor DNA compared to the same ratio in placental DNA as described previously (13). Tumors showing 5-fold or more increase in signals of given genes were considered to contain gene amplification.

**Results**

We studied 22 protooncogenes for amplification in DNA isolated from primary untreated tumor specimens of carcinoma of uterine cervix at clinical stages II or III. Twelve of the 50 (24%) of the tumor DNAs showed amplification of 5 or more copies with one or more probes studied. Amplification was observed in one case each (2%) with probes for the gene MYC/L1 (16 copies), SEA (13 copies), CCND1 (18 copies), BCLI (5 copies), and GLI (5 copies); in 2 cases each (2%) with HRAS (5 copies); and in 7 cases each (14%) with ERBB2 (5 to 68 copies) (Table 1; Fig. 1). In one tumor (tumor 59) CCND1 and SEA, which were mapped to the 11q13 region, were coamplified. ERBB2 also was amplified in the same tumor. Of the 7 tumors with ERBB2 amplification, 6 belonged to clinical stage III and 6 were of well differentiated histology, while one was moderately differentiated.

**Discussion**

The scope of gene amplification has not been well described in cervical cancer. In order to obtain such data, we evaluated the gene copy number of 22 protooncogenes in 50 tumor specimens obtained from primary, untreated cervical cancer patients. We found that ERBB2 was the most frequently amplified gene in this group of tumors. The genes MYC/L1, HRAS, SEA, CCND1, BCLI, and GLI were rarely amplified. In contrast to previous reports of studies from Europe and Mexico (4, 5), we did not find MYC to be amplified in this panel of tumors ascertained in India. The reasons for absence of...
MYC amplification in these tumors is unknown but may be attributed to geographic variations of etiology, such as type of HPV infection associated with tumor origin. ERBB2 encodes a 185,000 transmembrane protein which is highly homologous to the epidermal growth factor receptor gene which has been mapped to 17q21 (16). Activated ERBB2 has been shown to cause malignant transformation of cells in transfection assays (17). The ligand (gp30) for the transmembrane tyrosine kinase domain of ERBB2 has been identified which has been shown to inhibit growth of tumor cells which overexpress ERBB2 (18). Although amplification and/or overexpression of ERBB2 has been suggested to be an indicator of poor prognosis in breast, ovarian, and gastric carcinomas (10, 19, 20), its clinical relevance and prognostic significance has not been conclusively established. The ERBB2 gene has thus far not been reported to be amplified in squamous cell carcinomas. The frequency of ERBB2 amplification has been reported to vary in different tumor types and between different studies of the same tumor type (21). Some of this variation may be due to technical factors as well as the extent of stromal cell contamination in the tumor sample from which DNA was extracted.

It is interesting to note that 2 of the 7 tumors with amplified ERBB2 showed additional EcoRI restriction fragments. The sizes of these fragments were dissimilar in the two cases and were amplified severalfold (38 and 68 copies, respectively). They suggest mutations leading to either EcoRI polymorphisms or rearrangements. Previously novel EcoRI restriction fragments for ERBB2 were reported in breast and ovarian carcinomas (12).

Attempts to correlate ERBB2 amplification with histological progression and prognosis of different tumor systems have yielded inconclusive results (11, 22). Overexpression of ERBB2 was reported in over 60% of the patients with CIN grade 3 and invasive cancers (5). In the present study, 5 tumors showed >20-fold amplification of ERBB2, while the remaining 2 showed 5-fold amplification. In our series, all tumors showing amplification were moderate to well differentiated carcinomas. This is in contrast to the data of Adnane et al. (23) who reported ERBB2 amplification predominantly in poorly differentiated breast carcinomas. In this series, we investigated only advanced stage tumors. Therefore, it is important to study the ERBB2 amplification and its expression in various stages of CIN in order to understand its role and significance in cervical carcinogenesis and prognosis. The high frequency of ERBB2 amplification observed by us in carcinoma of uterine cervix and overexpression reported in CIN grade 3 (5) suggests that this alteration plays an important role in cervical carcinogenesis.

Acknowledgments

The authors thank the staff of the cancer clinic, Department of Obstetrics and Gynecology, LNJP Hospital, New Delhi, India, for providing tumor material. The authors also thank Professor Usha K. Luthra, Kuwait University, for her interest in the study.

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