Oncogene Alterations in Carcinomas of the Uterine Cervix: Overexpression of the Epidermal Growth Factor Receptor Is Associated with Poor Prognosis1

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ABSTRACT
The involvement of human papillomavirus (HPV) in the development of carcinomas of the uterine cervix has been firmly established. However, other genetic alterations also play an important role in the pathogenesis of cervical cancer. Therefore, we have investigated the role of several (onco)genes in cervical carcinoma.

In tumors from 136 patients with stage I and II cancer of the uterine cervix, the expression of epidermal growth factor receptor (EGFR), c-erbB-2/neu, p53, and murine double minute 2 (MDM-2) was studied using immunohistochemistry. In 32 cases, amplification of EGFR, c-erbB-2/neu, MDM-2, and c-myc was studied by Southern blot hybridization. The expression levels of these proteins were correlated with HPV positivity, International Federation of Gynecologists and Obstetricians stage, lymph node metastases, tumor diameter, vessel invasion, and disease-free and overall survival.

Moderate/strong expression of EGFR was observed in 43% of tumors. c-erbB-2/neu was focally positive in 12 cases. p53 showed moderate/strong expression in 32% of the tumors. Thirteen 66% of tumors showed a moderate/strong expression of MDM-2, and this expression was correlated to p53 expression (P < 0.001). Only moderate/strong expression of EGFR was associated with reduced disease-free (P = 0.002) and overall survival (P = 0.003). In multivariate analysis, the association of EGFR overexpression with poor prognosis was independent from lymph node status. Gene amplification was found for EGFR (four cases), c-erbB-2/neu (two cases), and c-myc (six cases). In two tumors, rearrangement of c-myc was found, probably due to the integration of HPV.

In conclusion, overexpression of the EGFR is an independent predictor for prognosis in earlier stages (stage I and II) of cervical cancer. p53 and MDM-2 expression are correlated to each other and may play a role in the interaction with HPV. The importance of c-erbB-2/neu and c-myc amplification is relatively small in stage I and II cervical cancer.

INTRODUCTION
Amplification of proto-oncogenes and inactivation of tumor suppressor genes play an important role in the pathogenesis of many different tumors. Studies in cervical carcinomas have shown that the EGFR, c-erbB-2/neu, and c-myc are important for the prognosis of patients with advanced tumors (stages II–IV), but the role for each of these genes in earlier stages (stages 0–I) is not yet clear.

The gene for the human EGFR is located on chromosome 7 and encodes a transmembrane cell surface glycoprotein of Mr 170,000 that binds epidermal growth factor, transforming growth factor-α, amphiregulin, and heparin-binding epidermal growth factor (1). Studies in different types of cancer have shown that increased expression of the EGFR generally is associated with a more aggressive biological behavior compared to tumors with low or normal expression (2). It has been reported that overexpression of the EGFR in cervical carcinomas correlates with a poor prognosis (3–5), although this association is controversial (6). Scambia et al. (6) found no relation of EGFR overexpression with prognosis.

The c-erbB-2/neu gene on chromosome 17q21 codes for a transmembrane M, 185,000 class I tyrosine kinase receptor protein structurally related to the EGFR (7, 8). The c-erbB-2/neu gene can be activated by point mutation or amplification. High expression of c-erbB-2/neu was associated with a poor prognosis in stages II and III for cervical cancer (9–12). Hale et al. (13) found that c-erbB-2/neu membrane expression was present in stages Ib and Ila, and this was also correlated with a poor prognosis.

The human p53 gene is located on chromosome 17p13.1. The p53 tumor suppressor is a multifunctional protein that plays a central role in the regulation of the normal cell cycle. Inactivation of p53 by mutation is a common event in the develop-

1 The abbreviations used are: EGFR, epidermal growth factor receptor; wt, wild-type; MDM-2, murine double minute 2; HPV, human papillomavirus; FIGO, International Federation of Gynecologists and Obstetricians.

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Oncogenes and Prognosis in Cervical Cancer

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The binding of wt and mutant p53. The Periodic acid-Schiff staining with diastase treatment and Alcian blue staining were used to assign tumors with mucin production and squamous morphology to the adenosquamous category. Maximum diameter of the primary tumor was determined according to the measurements taken during the pathology examination, and tumors were classified as ≤3 cm or ≥3 cm. If tumor cells were found in an endothelial lined space, lymphovascular space invasion was recorded. The number of mitoses was recorded as <15 per 10 high-power fields or ≥15 per 10 high-power fields at ×400 magnification and 0.4-mm field diameter.

Immunohistochemistry. Immunohistochemistry was performed on 5-μm sections using aminopropylethoxysilane-coated slides. Paraffin sections were deparaffinized and rehydrated, and endogenous peroxidase was quenched with 0.3% H2O2 in methanol for 20 min. Monoclonal antibodies directed against the following antigens were used: EGFR (E 30, 1:100; Biogenex, San Ramon, CA), c-erbB-2/neu (3B5, 1:20000; Ref. 23), p53 (Do-7, 1:500; DAKO, Glostrup, Denmark), and MDM-2 [IF-2 (AB-1), 1:800; Oncogene Science, Uniondale, NY]. Incubations were performed at room temperature. PBS with 1% BSA was used as diluent for all antibodies. Washing between incubations was performed three times for 5 min each in PBS. Prior to incubation with the primary antibodies to p53, c-erbB-2/neu, and Ki-67, the slides were subjected to antigen retrieval by submerging in a boiling solution of 0.01 M citrate buffer (pH 6.0) for 10 min (24) in a microwave oven at 700 W and cooled down in this buffer to room temperature for 2 h. For the antibody to MDM-2, 0.05% DET (DishClean) was added to the citrate buffer (25). Incubation with the EGFR antibody was preceded by pretreatment with 0.4% pepsin in 0.01 m HCl for 20 min at 37°C. After washing in PBS, slides were incubated overnight with the specific primary antibodies. Biotin-labeled rabbit antimouse immunoglobulins and a biotinylated horseradish peroxidase-streptavidin complex (both DAKO) were subsequently applied for 30 min each. A 0.05% solution of diaminobenzidine (Sigma Chemical Co., St. Louis, MO) with 0.0018% H2O2 in a 0.05 m Tris-HCl buffer (pH 7.6) was applied for 10 min to visualize the immune aggregates. For EGFR, 10 nm imidazole was added to the diaminobenzidine. Mayer’s hematoxylin was used for counterstaining of the slides.

Brown staining of the plasma membrane indicated positivity for EGFR and c-erbB-2/neu antibodies. Brown staining of the nucleus indicated positivity for p53 and MDM-2 antibodies. Omitting the primary antibody on serial slides served as a negative control. Appropriate positive control sections were stained simultaneously. For EGFR, normal cervical epithelium was used as a positive control; for c-erbB-2/neu and MDM-2, a breast carcinoma showing high expression was used as a positive control; and for p53, a colon carcinoma showing high p53 expression was used as a positive control.

The staining results for EGFR, p53, and MDM-2 were scored semiquantitatively. Scores representing the percentage of tumor cells stained positive were as follows: 0, no positive tumor cells; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 76–100%. Intensity was estimated in comparison to the control and scored as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. A final score was calculated by adding the scores for percentage and intensity, resulting in scores of 0 and 2–7. A score of 0 was deemed negative; 2–3 was...
considered weak; 4–5 was considered moderate; and 6–7 was considered strong. c-erbB-2/neu was scored as negative or positive. All slides were scored by two of us (A. M. F. K. or M. J. V. d. V.).

Southern Blot Analysis. DNA was isolated from frozen tissue of 32 tumor samples. DNA isolation was performed as described by Devilee et al. (26).

For Southern blot analysis, 15 μg of DNA were digested with the restriction enzyme EcoRI (Boehringer Mannheim, Mannheim, Germany), size-fractionated on a 0.8% agarose gel, and blotted onto nylon membranes (Hybond N+, Amersham, Amersham, United Kingdom). Membranes were prehybridized for 1 h at 65°C in hybridization mix (0.4 M Na₂HPO₄, 0.1 M NaH₂PO₄, 1 mmol of EDTA, and 0.25 M SDS). Insert fragments for the oncogenes EGFR (420-bp BamHI cDNA fragment; Ref. 27), c-erbB-2/neu (0.8-kb EcoRI cDNA fragment; Ref. 28), MDM-2 (585-bp reverse transcription-PCR fragment; Ref. 29), c-myc (exon 1, 554-bp fragment; Ref. 30), and HPV 18 (total HPV 18 genome; Ref. 31) were radiolabeled with 20 μCi of [α-³²P]dCTP (Amersham) using a random-primed labeling kit (Pharmacia, Uppsala, Sweden). After 16 h of hybridization, membranes were washed 30 min in 2× SSC/0.1% SDS, 30 min in 1× SSC/0.1% SDS, and 30 min in 0.5× SSC/0.1% SDS. Membranes were exposed to Kodak X-Omat AR films with intensifying screens at 70°C. Intensity of the bands was measured on a Molecular Dynamics (Sunnyvale, CA) PhosphorImager 445SI. Molecular Dynamics ImageQuant software was used for quantification of the products. Gene amplification was scored by comparing the hybridization results for different oncogenes to each other and to the positive controls taken along on the blots.

p53 Mutation Analysis (PCR-directed Sequencing). DNA isolation DNA from four primary tumors in paraffin-embedded sections was extracted according to the protocol described previously (32).

PCR. Screening for p53 gene mutations was performed for exons 5–8 by a PCR-directed sequencing method described below. This region was chosen because it contains the sites of >87% of p53 mutations found in various human cancers (14). The primers used for amplification were as follows: exon 5, sense 5'-TTCCTCTTACAGTACTC-3' and antisense 3'-TCTCTGCTGTCCCGACCAAC-5'; exon 6, sense 5'-TGGGGCTGGAGAGACGAC-3' and antisense 3'-AGGGATATTGGGGTACTCTACAC-5'; exon 7, sense 5'-TGGGCTGGAGAGACGAC-3' and antisense 3'-AGGGATATTGGGGTACTCTACAC-5'; exon 8, sense 5'-TGGGCTGGAGAGACGAC-3' and antisense 3'-AGGGATATTGGGGTACTCTACAC-5'. Primary tumor DNA was subjected to 47 cycles of “touch-down” PCR, with annealing starting at 65°C and decreasing to 57°C. All exons were amplified in 100 μl containing 10 ng of DNA, 20 pmol of each primer, 1 unit of SuperTaq polymerase (HT Biotech-
nology, Cambridge, United Kingdom), 4 mmol of dNTPs, and 10× SuperTaq polymerase buffer (containing 10 mmol of Tris, 1.5 mmol of MgCl₂, 50 mmol of KCl, 0.01% (w/v) gelatin, and 0.1% Triton).

Direct Sequencing. Double-stranded PCR products were sequenced using the PCR primers, as described previously. For nucleotide sequence analyses and comparisons, the programs Seqed and Fasta of the Genetics Computer Group (Madison, WI) sequence analysis software package (GenBank DNA data base, Version 8.1) were used.

HPV Detection and Typing. All 136 samples were initially tested by the CPI/CPII E2 consensus primers (33), which amplify a 188-bp fragment in the E2 open reading frame. β-Globin was coamplified in each reaction as a control using primers RS40/RS42 (198 bp; Ref. 34). Samples negative for HPV were subsequently tested by the GP5⁺/GP6⁺ L1 consensus primers (152-bp fragment in the L1 open reading frame; Ref. 35) and by the MY09/MY11 L1 consensus primers (450-bp fragment in the L1 open reading frame; Refs. 36 and 37). To determine the HPV subtype, the PCR products were subjected to direct sequence analysis. After purification of the PCR products using the EasyPrep kit (Pharmacia), 3 µl were sequenced directly with the cycle sequencing kit (Perkin-Elmer Corp., Norwalk, CT) using the [γ⁻³²P]ATP (Amersham) end-labeled PCR-primers. For nucleotide sequence analyses and comparisons, the programs Seqed and Fasta of the Genetics Computer Group sequence analysis software package (GenBank DNA data base, Version 8.1) were used.

Statistical Analysis. The different protein expression patterns were correlated among each other and to FIGO stage, lymph node positivity, tumor size, vaso-invasion, and histology. Correlations were evaluated with the χ² test. Disease-free and overall survival curves was calculated from the time of operation. Curves of the different expression patterns and clinicopathological parameters were made according to the Kaplan-Meier method. The curves were compared with the Gehan-Wilcoxon test. Multivariate analysis was performed using Cox’s regression model. A correction was made for multiple statistical testing by considering P < 0.01 significant. Results were considered marginally significant if 0.01 < P < 0.05.

RESULTS

Frequency and Type of HPV. HPV DNA was identified in 87% of the 136 tumors studied. HPV 16 was detected in 82 cases; HPV 18 was detected in 14 cases; HPV 31, 33, and 35 were detected in 11 cases; HPV 45 was detected in 5 cases; and HPV 51, 52, 58, 59, 68, and 73 were detected in 6 cases. In 18 tumors, no HPV could be detected with the three primer sets (CPI/CPII, GP5⁺/GP6⁺, and MY09/MY11) we used.

Protein Expression and Genetic Alterations. EGFR staining was moderate/strong in 73 cases and negative/weak in 63 cases. Many of the cervical carcinomas have a pattern in which solid nests of tumor cells are formed, sometimes with central keratinization. Usually the basal cells of these nests stained more intensely than cells in the middle of the nests (Fig. 1A). Normal epithelium always stained positive for the EGFR and was used as an internal positive control. Four of the 32 tumors studied had an amplification of the EGFR gene (Fig. 2). Of these four tumors, two showed moderate staining, one showed weak staining, and one showed no detectable staining. Frozen tissue of the last mentioned tumor showed moderate staining. The lack of a close correlation between gene amplification and staining results may be that amplification results in only a moderate increase in overexpression or because expression of EGFR is also disturbed by HPV-E5 and -E6 oncoproteins (38, 39).
Very focal c-erbB-2/neu staining was seen in 12 cases, all of the other samples were negative (Fig. 1D). Homogeneous positive staining was never observed. Two tumors with c-erbB-2/neu gene amplification were found, and both showed focal strong membrane staining of tumor cells (data not shown).

Staining of p53 was moderate/strong in 43 cases and negative/weak in 90 cases. p53 nuclear staining was mainly located at the basal site of the tumor nests, and less staining was present in the middle of the nests, comparable to the pattern found for EGFR staining (Fig. 1B). Dysplastic epithelium usually showed moderate/strong staining, whereas normal epithelium occasionally showed p53-positive cells. No correlation was found between p53 staining results and HPV positivity.

Only five tumors had a very strong expression of p53 detected with antibody DO-7. All five tumors were positive for HPV 16 or 18. Four of these tumors with a strong staining pattern for p53 were analyzed for mutations in exons 5–8. Two tumors containing HPV 16 showed a mutation in exon 7. The other tumors had no mutations in exons 5–8. These tumors may contain a mutation in one of the other exons of p53 or an other mechanism than mutation is the cause of the high expression of p53.

Seventeen cases showed a moderate/strong staining pattern for MDM-2, and 116 cases were negative/weak. The nuclear staining pattern was heterogeneous, whereas the normal epithelium was negative for MDM-2 (Fig. 1C). Correlation’s between the different staining patterns showed that p53 and MDM-2 staining were associated (P < 0.01). If MDM-2 showed stronger staining, p53 was also stronger. No MDM-2 amplifications were found in the 32 tumors studied.

c-myc was amplified in six tumors. In two of these tumors, which were HPV 18 positive, the EcoRI digestion showed the 12.5-kb germ-line band of c-myc and, in addition, a ~20-kb band. The HPV 18 probe hybridized with the same ~20-kb band and with the 7.9-kb band of HPV 18. In the HindIII digestion, again additional bands hybridizing with the c-myc probe were found, and these same bands hybridized to a HPV 18 probe. This indicates that HPV 18 is probably integrated near the c-myc gene (Fig. 3).

### Associations of Staining Results and HPV with Clinicopathological Parameters

The various protein expression patterns and HPV were correlated with FIGO stage, lymph node positivity, tumor size, vasoinvasion, number of mitosis, and histology. Table 1 shows the results of the correlation of staining patterns with the clinicopathological parameters. EGFR positivity was associated marginally with large tumor diameter (P = 0.03), FIGO stage II (P = 0.04), lymph node metastases (P = 0.03), and the squamous cell carcinoma histological type (P = 0.02). Positive c-erbB-2/neu staining correlates with the adenosquamous carcinoma histological type (P = 0.03). No statistically significant correlations could be found between p53 accumulation and any of the clinicopathological parameters. HPV positivity correlated strongly with large tumor diameter (P = 0.008) and with a high number of mitoses (P = 0.01).

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* The number of cases reported is affected by incidental missing cases.
Association of the Staining Results with Disease-free and Overall Survival. The follow-up period from the patients alive at last follow-up (n = 103) ranged from 1 to 133 months, with a median of 48 months.

Table 2 shows the results of the univariate analyses. Lymph node metastases, FIGO stage II, and a large tumor diameter were highly significant predictors for a shorter duration of disease-free and overall survival in the univariate analysis (P < 0.01). HPV-positive patients only have a shorter duration of overall survival (P < 0.01). Patients with a moderate/strong staining of the EGFR had a worse disease-free (P = 0.002) and overall survival (P = 0.003; Fig. 4). c-erbB-2 staining was a marginally significant predictor for a shorter duration of disease-free and overall survival in the univariate analysis (P = 0.03). Subsequently, multivariate analysis was performed using the Cox’s regression model. Included in the multivariate analysis for disease-free and overall survival were lymph node status, tumor size, FIGO stage, and EGFR staining. Lymph node status was, by far, the strongest prognostic factor for disease-free and overall survival. For overall survival, there was independent prognostic information added to the lymph node status by EGFR staining but not for FIGO stage and tumor size. Kaplan-Meier curves were made for overall survival of patients with negative/weak and moderate/strong EGFR staining patterns and positive or negative lymph nodes (P < 0.001; Fig. 5). For disease-free survival, statistically significant prognostic information was added to the lymph node status by tumor size but not for FIGO stage and EGFR staining (Table 3). When a margin of 4 cm was used for tumor size all associations with staining results and disease-free and overall survival were weakened.

### Table 2 Association of clinicopathological parameters and staining results with overall and disease-free survival

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</table>

<sup>a</sup> CPP, clinicopathological parameter; IHC, immunohistochemistry.
We have systematically studied the expression of various oncogene and tumor suppressor gene proteins in cervical carcinoma, stage I and II, in relation to clinicopathological parameters. Moderate/strong expression of the EGFR appeared to be associated with a poor prognosis in our study. Studies in different types of cancer, including cervical cancer (5), have shown that increased expression of the EGFR generally is associated with a more aggressive biological behavior compared to tumors with low or normal expression (2). In our study, this more aggressive behavior is expressed by the correlation of high EGFR staining with large tumor diameter (>3 cm) and lymph node metastasis, which is in agreement with other investigators (3, 5). Kristensen et al. (4), on the contrary, found no correlation with any of the histopathological variables: tumor size, grade of differentiation, vessel invasion, and lymph node metastasis. In the 26% of tumors that were positive, the relapse-free survival was lower for patients with tumors overexpressing the EGFR (4). The difference in percentage of positive tumors can be because Kristensen used another antibody with a different affinity. Furthermore, we also studied 28 FIGO stage II patients, whereas Kristensen investigated only stage Ib patients. We only found four tumors with amplification of the EGFR gene, of which three showed moderate staining and one weak staining. These results indicate that other mechanisms than amplification can also result in high expression of the EGFR. Crusius et al. (40) showed that the HPV-E5 oncogene enhances EGFR activation without up-regulating the number of receptors (39). The HPV-E6 oncogene gives higher mRNA levels of the EGFR and stabilization of the EGFR protein. In these cells, an enhanced transduction signal is measured (38). HPV-E5 gives also enhanced activation of c-erbB-2/neu, a protein that is structurally related to the EGFR (38). In our series, only 12 tumors with very focal overexpression of c-erbB-2/neu were found, even after antigen retrieval. Seven of these tumors were tested for amplifications, and only two showed an amplification of c-erbB-2/neu. Although the percentage of positively stained tumors was low, a shorter relapse-free and overall survival were found. It has been found that membrane expression of c-erbB-2/neu was more frequent in stages II and III than in stage I (9, 12). For all stages, tumors with high expression were associated with a poor prognosis (9–12). Kihana et al. (9) found that 11 of 44 adenocarcinomas of the cervix showed strong membrane-bound expression of c-erbB-2/neu; eight of these cases were shown to contain an amplification of the

**DISCUSSION**

| Relative risk factors for overall and disease-free survival analyzed with Cox’s regression model (n = 120 evaluable patients) |
|-------------------------------------------------|-----------------|-------------|
| Survival                                        | 95% confidence  | P           |
| Lymph node                                      |                 |             |
| Negative                                       | 1.00            |             |
| Positive                                       | 3.19 (1.48–6.88)| 0.003       |
| EGFR                                            |                 |             |
| Negative/weak                                   | 1.00            |             |
| Moderate/strong                                 | 2.73 (1.07–6.96)| 0.04        |
| Disease-free survival                           |                 |             |
| Lymph node                                      |                 |             |
| Negative                                       | 1.00            |             |
| Positive                                       | 3.77 (1.86–7.65)| <0.001      |
| Tumor size                                      |                 |             |
| ≤3                                             | 1.00            |             |
| ≥3                                             | 2.55 (1.16–5.62)| 0.02        |

**Table 3**

**Fig. 4** Kaplan-Meier curves for disease-free (a; P = 0.002) and overall (b; P = 0.003) survival of 136 patients with negative/weak and moderate/strong EGFR staining patterns.

**Fig. 5** Kaplan-Meier survival curve of 125 patients with negative/weak and moderate/strong staining patterns of the EGFR and positive or negative lymph nodes (P < 0.001)


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The low percentage of positive c-erbB-2/neu tumors in our study may be because we only studied FIGO stage Ib and Ila tumors. Furthermore, different antibodies with different affinities were used in the studies mentioned, and this can also be the cause of the differences in the percentage of c-erbB-2/neu-positive tumors.

p53 is also deregulated by the HPV oncoproteins, mainly by E6. E6 degrades p53 via the ubiquitin proteinysis pathway. On the basis of these findings, it is to be expected that tumors containing HPV DNA will contain low amounts of p53 protein. In contrast, 43 tumors (33 HPV positive) contained a moderate/strong staining pattern. This is in concordance with other investigators who found that overexpression of p53 protein is a common phenomenon in cervical cancer (16, 41–44). We have no explanation for the finding that p53 expression is moderate/strong in a high proportion of cervical carcinomas, including tumors containing HPV. We also did not find any correlation between moderate/strong p53 expression and HPV positivity, tumor subtype, lymph node status, and disease-free and overall survival (41–45). Mutated p53 has an extended half-life and can, therefore, be detected by immunohistochemistry. In many tumor types, the presence of p53 mutations is associated with immunohistochemical detectable p53 expression. We tested four tumors that had strong staining patterns for p53 mutations in exons 5–8, and two mutations were found. From published results on p53 mutations in cervical carcinoma, it can be concluded that mutations in the p53 gene are rare. The percentage of tumors with p53 mutations ranges from 0 to 10% (15, 17, 18, 46). In addition to p53 mutations, inactivation of p53 can also be the result of other mechanisms for instance binding of wt p53 by various oncoproteins (18). The MDM-2 gene encodes such an oncoprotein, which can bind to wt and mutant p53. The MDM-2 protein interaction domain covers the p53 activation domain required for activation of transcription, and this is the same domain bound by the HPV-E6 oncoprotein (47). MDM-2 overexpression inhibits transcriptional control by p53 protein by concealing the transactivation domain of p53 and results in the degradation of p53 (48, 49). We found only 17 cases with moderate/strong MDM-2 staining and a correlation between the expression of p53 and MDM-2. When MDM-2 was stronger, p53 was also stronger. These results indicate that the feedback loop is deregulated and that p53 is not degraded by MDM-2. Studies in human sarcomas, gliomas, breast cancer, and leukemias show that MDM-2 can be deregulated by amplification and overexpression (50–53). We did not find MDM-2 gene amplification in our series of cervical carcinomas, which is in agreement with findings by Kessis et al. (54) and Ikenberg et al. (55), who found 1 tumor with MDM-2 gene amplification in a total of 88 tumors.

Only six tumors with amplification of c-myc were found. We confirm that HPV can be integrated adjacent to the c-myc gene (56). Integration of HPV in the proximity of c-myc leads to overexpression and/or rearrangements of c-myc and deregulates its normal function. In tumors of the uterine cervix Ocadiz et al. (57) have shown c-myc amplification and/or rearrangement in 90% of 35 tumors in a Mexican population. Amplifications and/or rearrangements are frequently found but are more frequent in stages III and IV than in stages I and II of cervical cancer (58, 59).

In conclusion, we found that the moderate/strong expression of the EGFR is associated with poor prognosis in cervical cancer and that it adds independent prognostic information to the lymph node status. p53 and MDM-2 are correlated to each other and play a role in the interaction with HPV-E6. The importance of c-erbB-2/neu and c-myc amplifications is minor in stage I and II of cervical cancer. Further investigation is needed in different tumor stages for all of the genes studied, to validate our data, and to investigate in what stage which genes are activated.

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