Immunohistochemical detection of HPV proteins and c-erbB receptors in cervical lesion specimens from young women

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Summary

The intention of the present study was to assess immunohistochemically the expression of c-erbB receptor family in cervical carcinoma specimens of young women. Simultaneously, HPV was detected in the same specimens using immunohistochemical assays.

Seventy-five cervical intraepithelial and invasive cancer specimens were assessed retrospectively. Positive c-erbB-2 immunostaining was revealed in 11.7% of tumor specimens, while for c-erbB-3 and c-erbB-4 receptor the corresponding figures were 32% and 49.3%, respectively. Concerning HPV infection-markers, positive immunostaining was found in 31% of cases, while coilocytes were detected in 64% of patients.

The correlation of c-erbB receptor expression with malignant aggressiveness in cervical cancer cells concurs with similar data regarding other neoplasias, thus rendering these receptors an important predictive factor and future therapeutic target.

Key words: c-erbB over-expression; HPV; Cervical cancer; Immunohistochemistry.

Introduction

Cervical carcinoma remains one of the most prevalent malignancies of the female genital tract worldwide, despite that in many countries triage strategies based principally on the Papanicolaou smear, have helped in reducing both incidence as well as mortality rates. Numerous molecular and epidemiologic studies have proved conclusively HPV's role in cervical carcinogenesis: the association between HPV and cervical intraepithelial neoplasia is beyond doubt [1, 2]. HPV infection is considered causative to the subsequent development of cervical carcinoma through a gradual progression leading from mild intraepithelial lesions to more severe degrees of neoplasia that finally develop into invasive carcinoma [3].

HPV is a member of the papovavirus family, with more than 100 identified viral serotypes, almost 40 of which have been detected in the female genital tract. Classification is performed according to their potential to induce malignant transformation, into three categories. "High-risk" types are often detected in carcinomas and severe dysplasias, "intermediate-risk" types that are more usually detected in mild or severe dysplastic lesions rather than in carcinomas and a "low-risk" type subgroup [4]. Persistent infection with "high-risk" HPV is an essential, though not sufficient, prerequisite in the pathogenesis of cervical carcinomas. Cervical carcinoma is attributed mainly to types 16 and 18. However, the fact that many cases of HPV infection 'clear up' spontaneously and only a small percentage of affected women will finally develop cervical carcinoma, many years or decades later, implies that other factors as well do play a significant role in cervical carcinogenesis [5].

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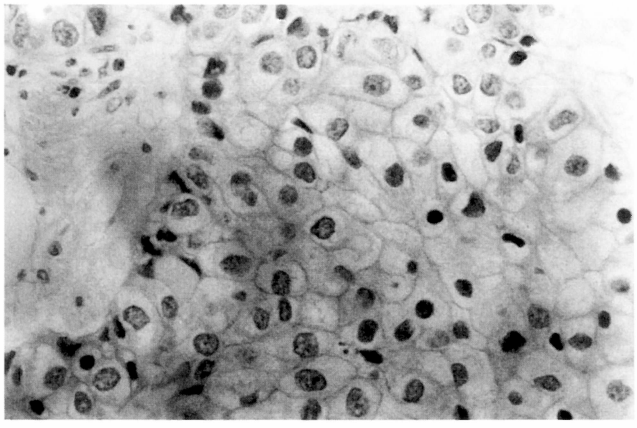
The HER family is a growth factor superfamily that includes c-erbB-1, B-2, B-3 and B-4 receptor proteins. All four are cell-membrane proteins with tyrosine kinase activity (also named RTK - receptor tyrosine kinase). Their activation finally results in diverse biologic responses, including proliferation, adhesion, cell motility, cell survival and programed cell death [1, 2, 6]. There are at least 11 known ligands for the HER family.

C-erbB-2, the only receptor that has no high-affinity ligand, is frequently detected in its activated form since it is the preferred heterodimerization partner for the other RTKs [7]. C-erbB-2 is overexpressed in numerous human cancers and has been shown to be an indicator of more aggressive disease and poorer prognosis in patients with cancers of the breast [8] while it is also overexpressed in some stomach and ovarian carcinomas [9, 10].

C-erbB-3 and c-erbB-4 are receptors activated by the neuregulin family of ligands, which is comprised of a number of different isoforms of NEU differentiation factor/Heregulin [11, 12]. C-erbB-3 is expressed in some breast and gastric carcinomas [13, 14], while little is known regarding its precise intracellular function. C-erbB-4 is the most recently identified member of the family, and is expressed in many adult and fetal tissue-lining epithelia of the gastrointestinal, urinary and reproductive tract, skin and nervous system [15] as well as in the majority of the ovarian cancers [16].

The purpose of the present study was the assessment of c-erbB-2, c-erbB-3 and c-erbB-4 immunoexpression in specimens of cervical carcinomas derived from young women, as well as the detection of human papilloma virus in the same specimens, using immunohistochemical assays.

Fig. 1



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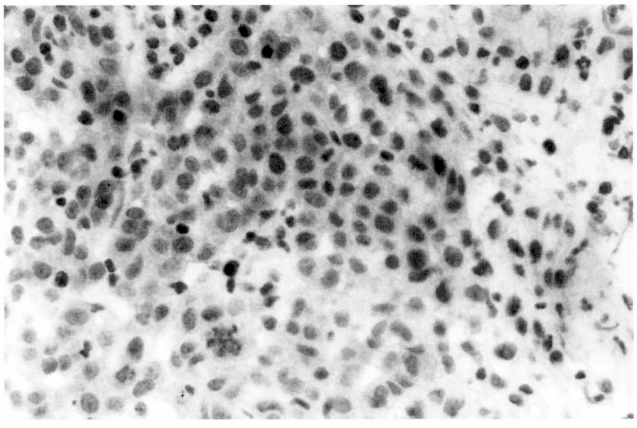
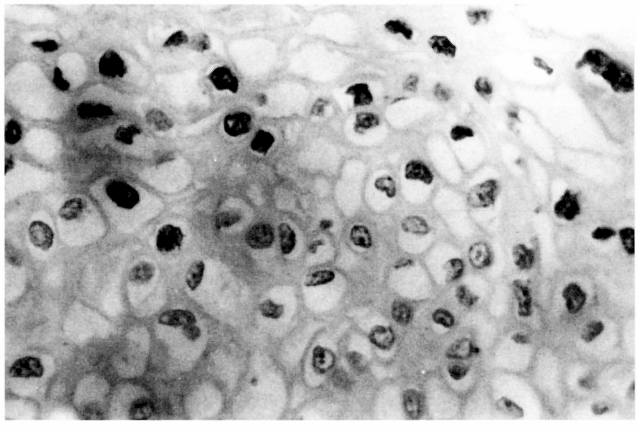


Fig. 3



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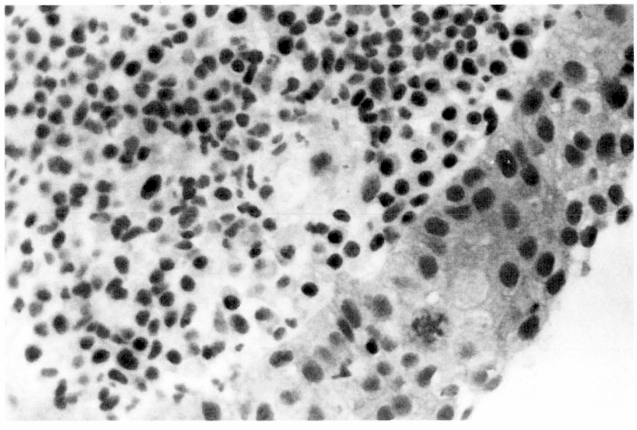

Figure 1. — Positive immunostaining for c-erb-B2 in an invasive cervical carcinoma.
Figure 2. — Positive immunostaining for c-erb-B4 in an invasive cervical carcinoma.
Figure 3. — Immunohistochemical detection of HPV in an in situ cervical carcinoma.
Figure 4. — Positive immunostaining for c-erb-B3 in an in situ cervical carcinoma.

Materials and Methods

Following identification of the cases from Patras University Hospital, specimens from 75 cervical lesions were studied retrospectively. The specimens derived from women younger than 40 years (median age 37 years) that had undergone surgery at the Gynecologic Department of Patras University Hospital. Previous surgery was either conization or abdominal hysterectomy (radical or total).

Histologic examination revealed invasive squamous cell cervical carcinoma in 42 cases while in the remaining 33 cases the diagnosis was in situ cervical carcinoma. All specimens were preserved in 10% formaldehyde and were constitutively embedded in paraffin.

The antibodies used for the immunohistochemical assay were: a) anti-HER-2 mouse monoclonal antibody (Biogenex), in a dilution 1:100, b) anti-HER-3 mouse polyclonal antibody (Santa Cruz Biothechnology Inc, UK) in a dilution 1:100 c) anti-HER-4 mouse polyclonal antibody (Santa Cruz Biothechnology Inc, UK) in a dilution 1:200, and d) anti-HPV mouse monoclonal antibody which detects type 1, 6, 11, 18, 16, and 31 of human papilloma virus. Negative staining controls were included in which no primary Ab had been added. As positive controls the peritumoral nonneoplastic cervical tissue was used.

At the same time the presence of coilocytes and multinucleated giant cells that constitute the morphological hallmark of HPV infection was detected in the tumor specimens.

Two skilled pathologists (D.K. and P.R.) scored all immunostains independently. The sections were whole mounted and examined with an OLYMPUS BX 4™ light microscope.

Immunohistochemical staining was graded on a scale of 0 to 1 according to the following assessment: 0 was attributed to < 5% positive cells; while 1+ was attributed to > 6% positive cells. For c-erb-B-2 protein only the membrane immunostaining was evaluated, while for c-erbB-3 and c-erbB-4 we evaluated either cytoplasmic or nuclear staining. The same scale of 0 (< 5%) and 1+ (> 5%) was used for all three receptors and for HPV. In the case of HPV the immunopositivity was only nuclear. The presence of coilocytic atypia was also observed in the intraepithelial as well as in the invasive carcinomas.

Statistical analysis of the results was performed using the SPSS-12 for Windows. The correlation between protein expression and the several clinicopathological factors was investigated by Pearson's chi square test. In cases where in the 2 x 2 crosstabs there was a count with a frequency less than 5% Fisher's exact test was used.

Results

Eight tumor specimens (corresponding to 11.7%) showed positive c-erbB-2 immunostaining. Of note is that they all derived from invasive carcinomas. Sixty specimens (80.0% of the total) showed no immunoreactivity. In the

remaining seven cases staining was ambiguous and therefore could not be evaluated. No c-erbB-2 immunoreactivity was detected in lesions classified as in situ carcinoma.

Regarding the c-erbB-3 protein, 24 cases with positive and 51 with negative immunoreaction were identified (32% and 68%, respectively). Of the cases with positive c-erbB-3 staining nine were related to in situ carcinomas, and 15 invasive carcinomas. Of the cases with negative c-erbB-3 staining 24 were related to in situ carcinomas and 27 to invasive lesions.

Thirty-seven (corresponding to 49.3%) of the tumor specimens were positive for the c-erbB-4 receptor, among which 15 involved in situ and 22 invasive carcinomas. Negative immunostaining was observed in the remaining 38 (50.7%), which was related to 16 in situ and 15 invasive carcinomas.

Regarding HPV infection-markers, positive immunostaining was found in 23 cases (12 in situ and 11 invasive) and negative in 45 tumor specimens (18 in situ and 27 invasive). Presence of coilocytes was observed in 48 (64% of the total) cases (24 in situ and 24 invasive lesions) while in the remaining 27 (36% of patients) no morphologic alterations of coilocytic atypia were observed (9 specimens concerned in situ and 18 invasive cancers).

The expression of c-erbB-2 was significantly correlated with the expression of c-erbB-3 ($p = 0.012$), as well as with that of c-erbB-4 ($p = 0.002$). The positivity of c-erbB-3 and c-erbB-4 receptors also had a strong association with one another ($p < 0.001$).

Positive immunostaining for HPV was found to have a statistically significant association with the expression of c-erbB-4 receptor ($p < 0.001$). No statistically significant correlation between HPV and either c-erbB-2 or c-erbB-3 immunoexpression was observed.

There was a statistically significant difference in the expression of c-erbB-2 between cases of in situ and invasive carcinomas ($p = 0.007$). Interestingly, no c-erbB-2 immunostaining was found in the tumor specimens derived from in situ carcinomas. No such correlation was observed for c-erbB-3 or c-erbB-4 proteins.

The presence of coilocytic atypia was strongly correlated, as expected, with the expression of HPV, however in 33 cases the presence of coilocytes was observed without positive immunostaining for HPV. Noteworthy was the significant association between the coilocytic alterations and the expression of c-erbB-3 and c-erbB-4 ($p = 0.017$ and $p < 0.001$, respectively).

Discussion

From the statistical analysis of the results, it is evident that the expressions of all three receptors have a strong association with each other, an observation that concurs with previous studies and is in line with existing knowledge regarding their intracellular interaction.

As other studies have indicated, there is overexpression of type 1 receptor tyrosine kinase (RTK), especially c-erbB-1 and c-erbB-2 in squamous cell cervical carcinomas [17]. Overexpression of c-erbB-2 has also been reported in the past to be present in more than 38% of patients with high grade squamous intraepithelial lesions (HGSIL) and invasive carcinoma [18].

Moreover, overexpression of the c-erbB-3 protein has been reported in 97% of cervical carcinomas, while positive staining of the c-erbB-4 protein has been found in 63% of squamous cell cervical carcinomas [18].

According to a study by Marmor *et al.* all receptor overexpression is correlated with cell proliferation and that conclusion agrees with our results [6]. The fact that the receptors cross-react and form heterodimers, via combined protein interactions concurs with our observation of simultaneous expression of the proteins. c-erbB-2 which acts as a coreceptor, following ligand activation of c-erbB-3 and c-erbB-4, heterodimerizes with these proteins forming the optimal signaling complexes for the initiation of the intracellular cascade. However, other authors have demonstrated a reduction of c-erbB-4 expression in some prostate and pancreatic carcinomas It is possible that each receptor induces different functions, and if both c-erbB-3 and c-erbB-4 are present, the relative expression levels of each, together with those of c-erbB-2 to which they heterodimerise, might determine the final cellular response.

The fact that we did not find c-erbB-2 overexpression in any of the in situ carcinoma specimens may indicate that this particular protein is not early activated in the malignant transformation process. We should also stress that in contrast to other studies, we used a monoclonal antibody that is very specific but not absolutely sensitive. Undoubtably immunohistochemistry cannot detect all cases of gene defects and overexpression, giving some false-negative results.

The statistically significant correlation between the presence of coilocytic atypia and the expression of c-erbB-3 and c-erbB-4 proteins should also be noted. We consider the coilocytes a more accurate indicator of HPV infection, thus we could support the idea of an intracellular interaction of the pathways in which RTKs and HPV are involved.

We have previously mentioned that we consider the presence of coilocytes a more precise marker of HPV infection than HPV-positive immunostaining. This is due to the fact that the anti-HPV antibody recognizes only types 1, 6, 11, 18 and 31 of the virus, while it is known that other types are also responsible for the malignant transformation process (such as "high-risk" types 33, 41 and 45).

Our study included only young women in whom the role of a sexually transmitted infectious factor may be more important. HPV infection occurs in sexually active women with a peak at the age of 27 years. Considering the long period of interval between infection and potent malignant transformation, cervical carcinoma is often found in women about 35-40 years (which were the ages included in our study). As long as the preventive and therapeutic HPV vaccines have not yet become widely available, and the existing therapeutic modalities are not suf-

ficiently effective in curing the disease, the cornerstone of prevention of cervical cancer will still be advocated by gynecologic-oncologists.

Conclusion

The results emerging from our study concur with current opinions considering the expression of the c-erbB superfamily as a reflection of the malignant potential of cervical cancer cells.

Complex intracellular pathway disruption, including the function of RTKs and their signals, may also contribute to the malignant process as our own study indicates and may become a potent future therapeutic target.

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