The Prognostic Significance of Human Epidermal Growth Factor Receptor Correlations in Squamous Cell Cervical Carcinoma

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Abstract. The aim of this study was to investigate the expression and prognostic influence of HER1 (EGFR), HER2 (c-erb-B2), HER3 (c-erb-B3) and HER4 (c-erb-B4) in squamous cell cervical carcinomas (SCC) and the importance of receptor correlations. Patients and Methods: 78 SCC were stained immunohistochemically for HER1-HER4. HER2 gene amplification was determined using fluorescence in situ hybridization (FISH). Parametric correlations were performed between the four receptors and tumor characteristics. Overall survival was evaluated by univariate and multivariate analyses. Results: Overexpression was found in 63% of SCC for HER1, in 21.8% for HER2, in 74.4% for HER3 and in 79.5% for HER4. Correlations were observed between HER1 and HER4 (p=0.019). Survival analyses revealed a significant association of HER1 overexpression with favorable outcome (p=0.016), while overexpression of HER2 and HER3 was associated with poor prognosis (p=0.006; p=0.05, respectively). HER1 remained significant in multivariate analysis. Conclusion: These data suggest that the prognostic relevance of the different HER receptors is influenced by the balance between the various receptors, especially of HER4.

Carcinoma of the uterine cervix remains the leading cause of cancer death in young women. In aggressive disease, a multimodal treatment approach includes platinum-based chemotherapy next to surgery and radiation, however, the efficacy of systemic treatment in this tumor entity is very limited (1). The identification of new therapeutic targets is therefore a major goal in improving patient outcome.

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The four members of the human epidermal growth factor receptor (HER) family: HER1 (EGFR, erbB1), HER2 (erbB/neu), HER3 (erbB3) and HER4 (erbB4) are transmembrane tyrosine kinases which take part in the regulation of cell proliferation, adhesion, migration and differentiation (2). Receptor activation and signal transduction act through homo- and heterodimerisation. The formation of various dimeric pairs is dependent on the affinity between the different receptors and the concentration of both ligands and receptors (3, 4). The specific pattern of the heterodimeric pairs seems to modulate the intracellular response. Receptor overexpression, especially of HER1 and HER2 has been associated with malignant potential and poor prognosis in various tumors (5).

These findings led to the development of specific antibodies targeting HER1 and HER2 for modern anticancer therapies (6). The development of pan HER inhibitors are hoped to further enhance the anti-tumor effects (7). In breast cancer as well as in other tumors, the anti-HER2 antibody trastuzumab (HerceptinTM,R) significantly improves survival, the strong anti-tumor activity being restricted to HER2-overexpressing tumours (8).

In carcinoma of the uterine cervix there are conflicting results regarding the frequency of HER2 expression, its prognostic consequence and consequently its value as a potential therapeutic target. Even more controversies exist regarding the role of the other EGF receptors in cervical cancer. This study, therefore, aims to investigate the expression pattern of all four EGF receptors, HER1-HER4, and the influence on prognosis focusing on squamous cell carcinomas of the uterine cervix.

Patients and Methods

Patients. This study included paraffin-embedded tumour samples of 78 patients diagnosed between 1987 and 1994 with primary invasive squamous cell cervical carcinoma. Age at diagnosis ranged from 24 to 89 years, with a median of 50 years. Clinical stage was Ia
Treatment consisted of surgery (radical hysterectomy +/- systematic lymph node dissection) in 39 patients, radiotherapy in 26 patients and surgery followed by radiotherapy in another 11 patients. In four patients, treatment was followed by platinum-based chemotherapy. Two patients did not receive any adjuvant treatment: a 84-year-old patient refused curative therapy on the basis of age, treatment was omitted for stage IV b disease in another patient due to impaired clinical status. Forty-one patients were classified as grade II and 37 as grade III. Median follow up for overall survival was 60 months (1-180 months).

**Immunohistochemistry (IHC) and Fluorescence in situ hybridisation (FISH).** For a detailed description of the methodology applied in this study please refer to Fuchs et al. (9).

Each slide was evaluated by two independent examiners who were blinded to the staining results of the partner proteins, the clinical data and the evaluation result of the second examiner.

For HER2, the official FDA scoring guidelines for predictive assessment in breast carcinoma were applied for the cervical carcinomas (HercepTest kit guidelines): 0 for no staining or membrane staining in less than 10% of the tumor cells; 1+ for only partial, weak staining of the cell membrane of more than 10% of the tumor cells; 2+ for moderate staining of the complete cell membrane in more than 10% of the tumor cells; 3+ for intense staining of the complete membrane in more than 10% of the tumor cells. HER2 overexpression was assessed as negative for scores of 0 or 1+ and positive 3+. For a score of 2+ the specimens were considered undetermined and these cases were, therefore, re-evaluated by FISH analysis.

HER1, HER3 and HER4 expression was analysed according to the immunoreactive score (IRS) described by Remmele and Stegner (10) and was assessed as overexpression for HER3 at a score >3 and for EGFR and HER4 at a score ≥6.

**Statistical analyses.** Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc.). Univariate analyses were assessed with $\chi^2$ and Fisher’s exact tests. Survival rates were assessed using survival curves according to Kaplan-Meier combined with log-rank-tests. For multivariate analyses, Cox regression models were performed. The level of significance was determined as $p<0.05$.

**Results**

Of the 78 tumors, a positive HER2 status was found in 17 cases (21.8%). Receptor overexpression was found in 49 cases (63%) for HER1, 58 cases (74.4%) for HER3 and in 62 cases (79.5%) for HER4. Correlation analysis was performed between the EGF receptors and between classical tumor characteristics (FIGO stage and grading). We observed direct correlations between overexpression of HER1 and HER4 ($p=0.019$). We did not find any significant correlations between the expression profile of any other EGF pairs or between HER1-4 and FIGO stage or grading (Table I).

HER1 overexpression was significantly associated with a better overall survival (Figure 1, $p=0.016$). The 5-year survival rate was 83.7% in HER1-positive patients compared to 58.6% in HER1-negative patients. These differences remained evident after controlling for stage ($p=0.01$) and grading ($p=0.01$).

HER2 overexpression in the carcinoma was significantly associated with poor overall survival (Figure 2, $p=0.006$). The 5-year survival rate was 47.1% in HER2-positive patients compared to 82% in HER2-negative patients. These differences remained significant after controlling for stage ($p=0.03$) and grading ($p=0.008$) in the univariate analysis.

HER3 overexpression was associated with poor overall survival just below reaching statistical significance ($p=0.05$, Figure 3). The 5-year survival rate was 69% in HER3-

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**Table I. Correlation between HER1-HER4 and tumour characteristics (n=78).**

<table>
<thead>
<tr>
<th></th>
<th>HER1</th>
<th>HER2</th>
<th>HER3</th>
<th>HER4</th>
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<tr>
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<td>0.066</td>
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<tr>
<td>Significance</td>
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<td>0.824</td>
<td>0.007</td>
<td>0.037</td>
<td>0.229</td>
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<tr>
<td>HER4</td>
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<td>0.745</td>
<td>0.570</td>
<td>0.582</td>
<td>0.050</td>
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<tr>
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<td>FIGO</td>
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**Figure 1. Overall survival according to HER1 overexpression. Patients with HER1-positive tumours (interrupted line) had a significantly longer overall survival than patients with HER1-negative tumours (continuous line).**
positive compared to 90% in HER3-negative patients. These differences were lost after controlling for FIGO stage ($p=0.42$) and grading ($p=0.065$).

HER4 overexpression itself did not show any statistically significant association with prognosis ($p=0.2$).

Multivariate analysis including FIGO stage and grading proved FIGO stage to be the most important prognostic factor, but confirmed HER1 to be associated with a good prognosis (Table II).

**Discussion**

In contrast to various other tumours, in cervical carcinoma specific antibody therapy targeting the HER family has not entered clinical practice. This is mainly due to the substantial disagreement regarding the rate of HER overexpression, the expression pattern of the individual receptors and ultimately their prognostic significance.

In preceding reports the HER2 overexpression rate ranged between 3% (11) and 77% (12). These large discrepancies have been attributed to methodological variations, differences in the threshold for the definition of HER2 overexpression as well as to diversities of the tumor histologies within the same study.

In order to overcome these obstacles, we focused on squamous cell carcinomas as a single histological tumor type and adopted the scoring system applied for the predictive HER2 assessment in breast cancer. HER2 positivity was stated in cases with strong HER2 overexpression or in cases with moderate expression and concomitant HER2 gene amplification: A positive HER2 status was found in 21.7%.

This rate is comparable with that observed in breast cancer. The results reflect the observation of previous studies in that firstly squamous cell carcinomas express lower HER2 levels than adenocarcinomas (12, 13) and secondly that the positive-rate is generally lower if a standardized scoring system is applied as compared to non-standardized analyses (11). The latter observation is underlined by the fact that in our study for HER1, HER3 and HER4 lacking an established evaluation system the overexpression rates clearly exceeded that of HER2. A very low HER2 expression rate of only 3% was described by Chaves Blanco et al. (10) in 35 primary cervical cancers. Since they used a similar methodology, the low expression rate might be due to the limited study size and their inclusion of various tumor types with unknown differentiation and grading.

It is noteworthy that in our study the various HER receptors had a different prognostic impact. Similar to the findings in breast cancer, HER2 and HER3 overexpression was associated with a poor prognosis (8, 9, 14). In contrast,
overexpression of HER1 was associated with a favorable prognosis. Though no prognostic impact was found for HER4 overexpression itself, we found a significant correlation between HER1 and HER4.

In the literature, the prognostic relevance of the EGF receptors is mainly based on the evaluation of HER1 or HER2 with - at first sight - contradictory results: several studies have associated both HER1 and HER2 with poor prognosis (15-18), others failed to show any prognostic effect (19, 20), or demonstrated astonishing results associating HER2 with a better outcome (21). Most of these studies did not analyze the other HER members. In those, however, which did, the prognostic impact of HER1 or HER2 was often dependent on the correlation with HER3 or HER4. Lee et al. found improved survival rates in a study on 55 cervical carcinomas overexpressing HER2 (22). The initial study was followed by a second study investigating HER1, HER3 and HER4 (22). They were able to demonstrate that the improved survival was associated with a strong correlation between HER2 and HER4. Memon et al. found in bladder cancer that an improved prognostic influence of HER1 and HER2 was restricted to cases showing a strong correlation with either HER3 or HER4 (23, 24). In breast cancer, several reports including our own series have demonstrated the favorable influence of HER4 on prognosis (25, 26). In breast cancer cells lines, HER4 showed pro-apoptotic functions (27). In addition, transfection of HER4 into HER2-overexpressing cells results in anti-proliferative, differentiating responses suggesting that HER4 signaling has an antagonistic effect on HER2 signaling (27).

Conclusion

Firstly, all four EGF receptors can be found in squamous cell carcinomas of the uterine cervix. Secondly, the rate of a positive HER2 status in this tumor entity seems to be similar to that found in breast cancer. Thirdly, the specific function of the different EGF receptors and consequently their prognostic relevance is closely connected to the expression profile of the other HER members, especially that of HER4. Transferring these observations into clinical practice, squamous cell carcinoma of the uterine cervix represents a worthwhile target for directed anti-HER antibody therapy. However, it becomes increasingly evident that not only the single growth factor receptor, but also the expression profiles of the individual HER members, especially of HER4, should be taken into consideration. Further prospective studies on the prognostic effects of HER receptor combinations are urgently needed.

Acknowledgements

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References


