Original Paper

ORL

ORL 2004;66:21–26
DOI: 10.1159/000077229

Received: July 28, 2003
Accepted after revision: January 29, 2004

Pharmacodiagnostic Value of the HER Family in Head and Neck Squamous Cell Carcinoma

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Key Words
EGFR · HER2 · Pharmacodiagnostics, head and neck cancer

Abstract
Two protooncogene products, EGFR (Her-1, c-erbB-1) and HER2 (Her-2/neu, c-erbB-2), have been reported to be frequently overexpressed in head and neck squamous cell carcinoma (HNSCC). In order to identify patients who may benefit from targeted cancer treatment for these two molecules, we determined the expression status of EGFR and HER2 in 129 HNSCC tumor specimens. Two pharmacodiagnostic kits (EGFR pharmDx™ and HercepTest™) were used to identify HNSCC tumors that overexpress EGFR or HER2. Overexpression of EGFR was detected in 42.6% of the tumor specimens, while HER2 was only rarely expressed (overexpression was observed in just 3.1% of all cases). Given the necessity of new therapeutic modalities for patients suffering from HNSCC, treatment EGFR signaling inhibitors appears to be warranted, whereas therapeutic intervention with HER2 inhibitors seems to be inappropriate in this tumor type.

Introduction

The superfamily of cell surface tyrosine kinase receptors plays an important role in fundamental cellular processes, including cell cycle progression, migration, metabolism, proliferation, differentiation and survival. Many human tumors express high levels of growth factor receptors, raising the possibility that receptor blockade may be useful as a cancer treatment strategy [1]. The HER growth receptor family consists of 4 cell surface receptors, including the epidermal growth factor receptor (EGFR, c-erbB-1 or HER1), c-erbB-2 (HER2/neu), c-erbB-3 (HER3) and c-erbB-4 (HER4) [2]. EGFR, HER2 and HER4 possess intrinsic tyrosine kinase activity, whereas HER3 does not. All receptors of the HER family can function synergistically with one another. Receptor activation results in the phosphorylation of specific tyrosine residues within the cytoplasmic region, which leads to simultaneous stimulation of multiple signaling pathways.

EGFR overexpression has been observed in human cancers including breast, ovarian, prostate, bladder, lung, brain and pancreatic cancer [3]. EGFR expression in head and neck squamous cell carcinoma (HNSCC) has been reported in approximately 90% of specimens; it is associ-

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0301–1569/04/0661–0021$21.00/0

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ated with poor prognosis [4, 5]. The HER2/neu oncoprotein has been most extensively characterized in breast cancer, where its expression correlates with poor prognosis and resistance to chemotherapeutic agents [6, 7]. HER2 overexpression in HNSCC has been described previously [8–10]. However, reports on the role of HER2 in HNSCC are less conclusive, as HER2 has been described to be overexpressed in very few investigated HNSCC specimens and because the correlation with clinical parameters is controversial [8–13].

The critical role that EGFR and HER2 play in cancer has led to an extensive search for selective inhibitors of their signaling pathways. The results of a large body of clinical trials thus far conducted suggest that targeting the HER receptor family could represent a significant contribution to improvement of HNSCC therapy [14]. The most promising strategies in clinical development include: (1) immunotherapy with monoclonal antibodies (mAb) to prevent ligand binding, and (2) targeted chemotherapy with small molecule inhibitors of the tyrosine kinase enzymatic activity to inhibit autophosphorylation and downstream intracellular signaling of the HER receptor family [15, 16]. However, optimal use of this type of targeted cancer therapy requires quantitative determination of the EGFR and HER2 status in every patient treated, since the presence of receptor overexpression is the sole eligibility criterion for treatment with target-specific therapeutic agents. In order to identify those patients who may benefit from targeted cancer therapy with EGFR and HER2 signaling pathway inhibitors, we used semiquantitative immunohistochemistry to determine the EGFR and HER2 expression status in tissue specimens of 129 HNSCC patients.

Materials and Methods

One hundred and thirty-seven potential candidates for targeted cancer therapy fulfilled the inclusion criteria of histopathologically confirmed squamous cell carcinoma of the pharynx or oral cavity and cancer therapy at the Department of Otorhinolaryngology of the University Hospital Innsbruck between 1997 and 2002. The following details were recorded: patient gender, age at tumor resection, site, stage, grade, therapy and day of death. The majority of the survival data was provided from the local cancer registry of the federal state of Tyrol.

Formalin-fixed, paraffin-embedded tissue blocks containing sections of primary tumor were available from 129 patients. Specimens were recut and deparaffinized in 2 sequential xylene baths (5 min), 100% ethanol (3 min) and 95% ethanol (3 min), followed by a 5-min washing step in water. Thereafter, semiquantitative pharmacodiagnostic kits were used according to the manufacturers' guidelines.

To identify tumors that overexpress EGFR, stainings were performed with EGFR pharmDx™ test (DakoCytomation, Glostrup, Denmark). All reagents were included in the kit and the staining protocol was followed minutely. Briefly, following washes and incubations were applied sequentially: 5-min wash using wash buffer, 5-min proteinase-K incubation, 5-min wash, 5-min peroxidase blocking agent incubation, 5-min wash, 30-min incubation with primary anti-EGFR mAb, clone 2-18C9, 5-min wash, 30-min incubation with HRP-labeled goat anti-mouse Ig polymer, 2 × 5-min wash, 10-min incubation using DAB substrate, 5-min wash, 1-min counterstaining with hematoxylin, 5-min gentle rinse in water.

HercepTest™ (DakoCytomation) was used to identify HER2-overexpressing tumors. Stainings were performed after rehydration of slides in strict adherence to the manufacturer's protocol as follows: 60-min incubation at 99°C in epitope retrieval solution, 20-min cooling down at RT, 5-min wash in wash buffer, 5-min peroxidase blocking agent incubation, 5-min wash, 30-min incubation with rabbit anti-HER2 pAb, 5-min wash, 30-min incubation with HRP-labeled goat anti-rabbit Ig polymer, 2 × 5-min wash, 10-min incubation using DAB substrate, 5-min wash, 1-min counterstaining with hematoxylin, 5-min gentle rinse in water. Finally, the slides were mounted using faramount aqueous mounting medium (Dako).

Using a light microscope, slide evaluation was performed by one author (V.H.S.) and by a pathologist. All slides were scored and recorded without knowledge of the patient's outcome. For EGFR pharmDx the intensity of the immunohistochemical staining was scored using the four-tier system described in the manufacturer's guidelines (0 = no staining; 1 = weak; 2 = moderate; 3 = strong). The percentage of positive-stained tumor cells represented the proportion score (0 = none; 1 = <10%; 2 = 10–50%; 3 = 50–80%; 4 = >80%). The total score was calculated by multiplying the intensity with the proportion score. A total score >4 was defined as overexpression. The staining pattern achieved with HercepTest was categorized as 0 (none or staining in <10% of tumor cells), 1+ (weak membrane staining in >10% of tumor cells), 2+ (moderate staining in >10%) and 3+ (strong staining in >10%) as recommended by the manufacturer.

Statistical analyses were done with SPSS software. The relationship between the expression of markers and the clinical parameters was calculated with the χ^2 test. Survival rates were calculated with the Kaplan-Meier method and analyzed with the log rank test.

Results

Of the 129 patients tested the EGFR and HER2 overexpression, 109 patients had first, 9 had second and 4 third head and neck carcinoma. For the other 7 patients, only recurrent data were available for the observed time frame. Tumors were either located in the pharynx (96 specimens) or in the oral cavity (33 samples). One hundred and six men and 22 women with a median age of 57.2 (37–92) years were assigned to 4 groups according to the UICC classification guidelines: 11 in stage I, 9 in stage II, 12 in stage III and 97 cases in stage IV. Patient characteristics are listed in table 1.

Table 1. Patient characteristics (n = 129)

Sex	
Male	106
Female	22
Not known	1
Age at diagnosis, years	
Mean	58.2
Median	57.2
Range	37–92
Tumor site	
Hypopharynx	6
Oropharynx	88
Nasopharynx	2
Oral cavity	33
Tumor size	
T1	20
T2	25
T3	12
T4	72
Nodal stage	
N0	40
N1	22
N2	51
N3	14
Nx	2
Metastases	
M0	114
M1	7
Mx	8
Stage	
I	11
II	9
III	12
IV	97
Grading	
G1	7
G2	73
G3	33
G4	4
Gx	12
Alcohol consumption[1]	
None, occasionally	55
Daily, alcohol abuse	46
Not known	28
Nicotine consumption[1]	
None, light smoker	31
Moderate or heavy smoker	81
Not known	17

[1] Patient information.

All patients except 1 underwent a surgical tumor resection or combined radio-chemotherapeutical treatment or both, depending on staging and compliance. So far, 94 patients have completed their recommended therapy (table 2) while 35 are either still receiving therapy or have dropped out.

All sections remained adherent and background staining or artifacts were not observed. Strong and complete membrane staining with occasional cytoplasmic reactivity (considered a 3+ score) was detected in 52 samples stained with EGFR pharmDxE kit. The results of this immunohistochemical examination are summarized in table 3. A moderate, complete or incomplete membrane staining (2+ score) was observed in 21 samples, a weak (1+) intensity in 18 and no reaction was observed in 38 cases. A total score >4 was observed in 55 specimens (42.6%).

Similarly, HER2 reactivity was determined in all 129 specimens using the FDA-approved HercepTest. A weak (1+) reaction was observed in 18, a moderate (2+) in 1 and a strong (3+) in 3 cases, whereas no signal was detected in the remaining 107 cases. Only 3.1% of all cases were rated as overexpressing HER2 according to the manufacturer's recommendation.

Another field of interest was the possible correlation between overall survival and EGFR or HER2 expression in particular tumor specimens. These analyses yielded no

Table 2. Therapy of 94 patients

Surgical therapy		
Surgery (primary site)		7
Neck dissection		6
Surgery (primary site + ND)		72
None		9
Radiochemotherapy	1st cycle	2nd cycle
Combined adjuvant	31	53
Combined neoadjuvant	30	–
Combined palliative	13	14
Radiation	8	4
Chemotherapy	1	2
None	11	21

ND = Neck dissection.

Table 3. Expression of EGFR and HER2 in 129 HNSCC specimens

Stage	EGFR pharmDX				Total score >4	HercepTest			
	0	1+	2+	3+		0	1+	2+	3+
I	–	6	–	5	5	10	1	–	–
II	4	–	2	3	3	9	–	–	–
III	5	2	3	2	3	11	1	–	–
IV	29	10	16	42	44	77	16	1	3
Total	38	18	21	52	55	107	18	1	3

statistically relevant data either for EGFR positivity or EGFR overexpression (fig. 1a, b). On the contrary, the presence of HER2 in tumors was significantly correlated with reduced overall survival of patients (fig. 1c).

Discussion

It has been repeatedly demonstrated that the good results achieved by surgical and radiotherapeutic treatment in early-stage HNSCC cannot be matched when applied to advanced disease. Therefore the current approach to the treatment of advanced HNSCC is a combination of tumor resection, radiotherapy and chemotherapy. Since chemo- and radiotherapy generally do not discriminate between normal and malignant tissues, radiochemotherapeutic regimens in HNSCC patients have been found to be associated with significant toxicity. Such cytotoxic schedules generally show poor outcome in terms of disease control and overall survival, and have not improved over the last 20 years [17]. These sobering facts underscore a need for new approaches in the treatment of HNSCC that demonstrate efficacy in targeting the tumor while limiting the damage to healthy cells. Cancer treatment targeted at the growth factor receptors is one such approach that has shown promising results for the management of a broad range of tumors.

Pharmacodiagnostics should provide oncologists with reliable and rapid information whether a patient can benefit from tumor-specific therapy with a particular mAb or synthetic inhibitor. For this purpose, it is necessary to determine the expression status of the tumor-associated target for each individual patient. In this study we used two immunohistochemical pharmacodiagnostic kits to determine the EGFR and HER2 expression status in a population of HNSCC patients.

EGFR was detected in 70.5% of the samples, and EGFR overexpression was observed in 42.6% of the investigated HNSCC specimens and with the highest frequency in advanced tumors. Since there are no accepted guidelines for the pharmacodiagnostic evaluation of the EGFR expression status, overexpression was calculated as the product (total score) of the four-tier intensity score and the proportion score [18, 19]. In our study, a total score >4 was evaluated as EGFR overexpression. We suggest that the criteria described are suitable for preliminary studies, but a definitive EGFR scoring system and the threshold for a positive result have to be determined on the basis of the clinical response to EGFR-targeted therapy in clinical trials.

EGFR expression in primary tumors was found to predict clinical outcome independently of cervical lymph node status, currently the most accurate prognostic parameter in HNSCC [4]. Moreover, EGFR overexpression has been reported to be a highly significant and independent indicator of prognosis in HNSCC patients; EGFR protein levels in primary head and neck tumors were also found to be associated with decreased disease-free and cause-specific overall survival [20, 21]. In disagreement with these reports, we did not observe a significant association between EGFR overexpression and disease progression in our patient cohort. After a thorough comparison between previously published data and our results, we conclude that this discrepancy may be associated with a higher overall survival rate of the patients in our study and a difference in the methodological approach for detecting EGFR expression. From a pharmacodiagnostic point of view, however, the tumor specimens obtained from nearly 50% of HNSCC cases overexpressed EGFR, and these patients appear to be eligible for treatment with EGFR-targeted cancer therapy.

HER2 was only expressed in 17% of HNSCC tumors and an overexpression was detected in just 3.1% of speci-

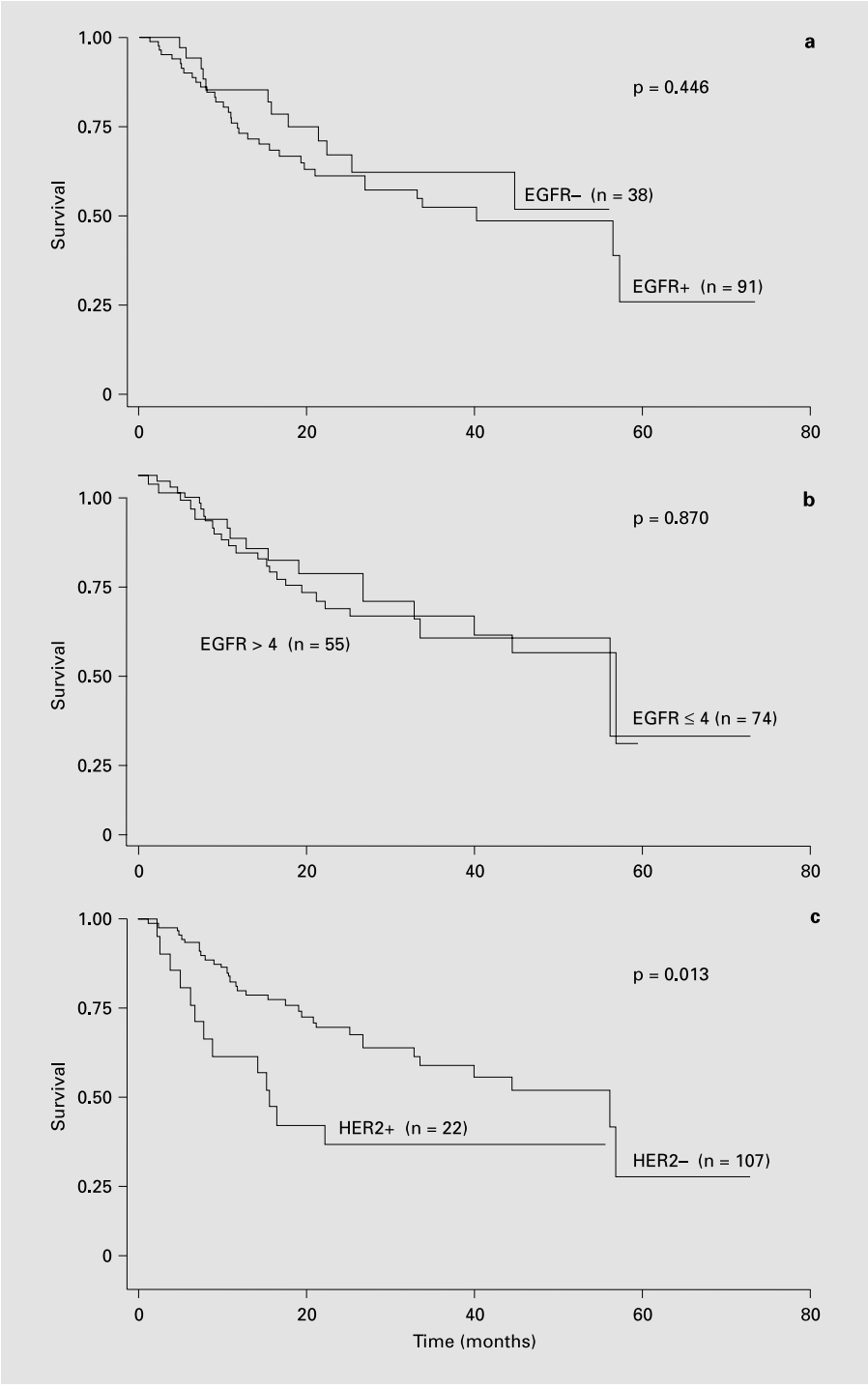

Fig. 1. Kaplan-Meier analysis showing the overall survival of 129 HNSCC patients in relation to EGFR expression (**a**), EGFR overexpression (EGFR score >4) vs. non-overexpression (EGFR score ≤4) (**b**) or HER2 expression status in the tumor specimen (**c**).

mens. These data contrast with a previous report that demonstrated HER2 overexpression in 47% of HNSCC specimens [10]. However, different anti-HER2 mAb and avidin-biotin-based immunoperoxidase detection systems were used in the mentioned study. These controversial data support the necessity of standardization and usage of validated immunohistochemical stainings for pharmacodiagnostic purposes.

Furthermore, it has been shown previously that of all the members of the HER family, HER2 was the most sig-

nificant factor in predicting outcome in HNSCC patients [22]. In our patient cohort, a statistically significant correlation between HER2 expression and decreased overall survival was demonstrated ($p = 0.013$). On the other hand, only a small proportion of tumors obtained from HNSCC patients (3%) overexpressed HER2. These data clearly show that even though HER2 may be a good prognostic marker for HNSCC, the therapeutic intervention with HER2-blocking agents might only play a marginal role in HNSCC due to the low incidence of suitable patients for this type of targeted cancer therapy.

In conclusion, we assume that a large proportion of patients suffering from HNSCC may be considered candidates for treatment with EGFR-specific inhibitors. By contrast, therapeutic intervention with trastuzumab or other inhibitors of the HER2 signaling pathway seems to be inappropriate in this type of tumor. Finally, this study demonstrates the necessity of rethinking the design of clinical analyses performed for pharmacodiagnostic reasons, which may run counter to the objectives of the prognostic clinicopathological studies.

Acknowledgments

This work was supported by grants P14933 and P16987 from the Austrian Science Fund (L.K., M.W. and G.M.S.), by a grant from the Tyrol Science Fund for Medical Research 50a (V.H.S. and I.B.) and from The Austrian Cancer Society – Tyrol (J.A.).

References

1 Lango MN, Shin DM, Grandis JR: Targeting growth factor receptors: Integration of novel therapeutics in the management of head and neck cancer. Curr Opin Oncol 2001;13:168–175.
2 Carpenter G, Cohen S: Epidermal growth factor. J Biol Chem 1990;265:7709–7712.
3 Gullick WJ: Prevalence of aberrant expression of the epidermal growth factor receptor in human cancers. Br Med Bull 1991;47:87–98.
4 Grandis JR, Melhem MF, Gooding WE, Day R, Holst VA, Wagener MM, Drenning SD, Tweardy DJ: Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 1998;90:824–832.
5 Ke LD, Adler-Storthz K, Clayman GL, Yung AW, Chen Z: Differential expression of epidermal growth factor receptor in human head and neck cancers. Head Neck 1998;20:320–327.
6 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–182.
7 Torre EA, Salimbeni V, Fulco RA: The erbB 2 oncogene and chemotherapy: A mini-review. J Chemother 1997;9:51–55.
8 Craven JM, Pavelic ZP, Stambrook PJ, Pavelic L, Gapany M, Kelley DJ, Gapany S, Gluckman JL: Expression of c-erbB-2 gene in human head and neck carcinoma. Anticancer Res 1992;12:2273–2276.
9 Field JK, Spandidos DA, Yiagnisis M, Gosney JR, Papadimitriou K, Stell PM: C-erbB-2 expression in squamous cell carcinoma of the head and neck. Anticancer Res 1992;12:613–619.
10 Hoffmann TK, Ballo H, Braunstein S, Van Lierop A, Wagenmann M, Bier H: Serum level and tissue expression of c-erbB-1 and c-erbB-2 proto-oncogene products in patients with squamous cell carcinoma of the head and neck. Oral Oncol 2001;37:50–56.
11 Chiesa F, Mauri S, Tradati N, Calabrese L, Giugliano G, Ansarin M, Andrle J, Zurrida S, Orecchia R, Scully C: Surfing prognostic factors in head and neck cancer at the millennium. Oral Oncol 1999;35:590–596.
12 Kearsley JH, Leonard JH, Walsh MD, Wright GR: A comparison of epidermal growth factor receptor (EGFR) and c-erbB-2 oncogene expression in head and neck squamous cell carcinomas. Pathology 1991;23:189–194.
13 Khan AJ, King BL, Smith BD, Smith GL, DiGiovanna MP, Carter D, Haffty BG: Characterization of the HER-2/neu oncogene by immunohistochemical and fluorescence in situ hybridization analysis in oral and oropharyngeal squamous cell carcinoma. Clin Cancer Res 2002;8:540–548.
14 Ford AC, Grandis JR: Targeting epidermal growth factor receptor in head and neck cancer. Head Neck 2003;25:67–73.
15 Herbst RS, Hong WK: IMC-C225, an anti-epidermal growth factor receptor monoclonal antibody for treatment of head and neck cancer. Semin Oncol 2002;29:18–30.
16 Kim ES, Kies M, Herbst RS: Novel therapeutics for head and neck cancer. Curr Opin Oncol 2002;14:334–342.
17 Vokes EE, Weichselbaum RR, Lippman SM, Hong WK: Head and neck cancer. N Engl J Med 1993;328:184–194.
18 Goldstein NS, Armin M: Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: Implications for a standardized scoring system. Cancer 2001;92:1331–1346.
19 Spizzo G, Obrist P, Ensinger C, Theurl I, Dunser M, Ramoni A, Gunsilius E, Eibl G, Mikuz G, Gastl G: Prognostic significance of Ep-CAM and Her-2/neu overexpression in invasive breast cancer. Int J Cancer 2002;98:883–888.
20 Charoenrat P, Rhys-Evans P, Modjtahedi H, Eccles S: The role of c-erbB receptors and ligands in head and neck squamous cell carcinoma. Oral Oncol 2002;38:627.
21 Putti TC, To KF, Hsu HC, Chan AT, Lai GM, Tse G, Lee YS, Whang-Peng J, Millward M, Lin L, Lin X, Lee CS: Expression of epidermal growth factor receptor in head and neck cancers correlates with clinical progression: A multicentre immunohistochemical study in the Asia-Pacific region. Histopathology 2002;41:144–151.
22 Xia W, Lau YK , Zhang HZ, Xiao FY, Johnston DA, Liu AR, Li L, Katz RL, Hung MC: Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. Clin Cancer Res 1999;5:4164–4174.