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Correlation between human epidermal growth factor receptor family (EGFR, HER2, HER3, HER4), phosphorylated Akt (P-Akt), and clinical outcomes after radiation therapy in carcinoma of the cervix ☆

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Abstract

Objective. To investigate prognostic significance of and correlations between HER1 (EGFR), HER2 (c-erb-B2), HER3 (c-erb-B3), HER4 (c-erb-B4), and phosphorylated Akt (P-Akt) in patients treated with radiation for cervical carcinoma.

Methods. Fifty-five patients with stages I–IVA cervical carcinoma were treated with definitive radiotherapy. Tumor expression of each biomarker was quantitatively scored by an automated immunohistochemical imaging system. Parametric correlations were performed between biomarkers. Univariate and multivariate analysis was performed with disease-free survival (DFS) and overall survival (OS) as primary endpoints.

Results. Correlations were observed between expression of HER2 and HER4 ($P = 0.003$), and HER3 and HER4 ($P = 0.004$). Decreased HER2, HER4, and P-Akt expressions were significant for diminished DFS on univariate analysis ($P = 0.04$, $P = 0.008$, and $P = 0.02$, respectively). Increased EGFR, and diminished HER2, HER4, and P-Akt expression were significant or showed trends toward significance for diminished OS on univariate analysis ($P = 0.07$, $P = 0.008$, $P = 0.09$, and $P = 0.08$, respectively). After controlling for pretreatment factors, multivariate analysis revealed HER2 associated with improved OS ($P = 0.05$).

Conclusions. These data emphasize that significant correlations exist between the differential expression of various HER family receptors. Multivariate analysis revealed only increased HER2 expression associated with improved OS after controlling for pretreatment clinical factors. These data emphasize the importance of continued basic and translational research on the HER family of receptors in cervical carcinoma.

Keywords: EGFR; HER2; HER3; HER4; Cervix cancer

Introduction

The human epidermal growth factor receptor (HER) family plays a key role in regulation of mammalian cell survival, proliferation, adhesion, and differentiation [1–4]. This HER family of receptor tyrosine kinases comprises four structurally related transmembrane receptors: HER1 (EGFR or erbB1), HER2 (HER2neu or c-erb-B2), HER3 (c-erb-B3), and HER4 (c-erb-B4). All members of the family have an extracellular ligand-binding domain, a transmembrane domain, and a cytoplasmic tyrosine kinase domain which for HER3 is nonfunctioning [1,5]. In response to ligand specific binding, these receptors act by forming hetero- or homodimers which thereby initiate tyrosine kinase activity in the intracellular domain. This amplification of tyrosine kinase activity promotes the phosphorylation of several tyrosine residues in the C-terminus which leads to a complex signaling cascade. This signaling cascade has been

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shown to result in cell proliferation, differentiation, migration, adhesion, protection from apoptosis, and transformation [1,5,6].

The oncogenic pathway of some cells is thought to be initiated as a result of HER family receptor mutation, overexpression, structural rearrangements, and/or relief of normal regulatory or inhibitory pathways [1,4–7]. The presence of EGFR and Her2neu receptors has also been associated with accelerated tumor progression and resistance to therapy for multiple types of malignancies [1,5,6] and small molecule inhibitors of their activity are being examined as targeted therapy in cancer patients. In addition, overexpression of all four receptors has been observed in malignancies such as breast cancer [4,5]. Of note, HER4 has in multiple reports been linked to a good prognosis and a longer disease-free interval [3,4,8]. The causal relationship of this receptor network to disease progression and resistance to therapy provides a rationale for targeting this signaling system with tumor-selective strategies.

The phosphatidylinositol 3-kinase (PI3K) family of enzymes has also been well studied with respect to promotion of cellular growth and survival in cancer cells [6,9,10]. These kinases can be activated from cell surface growth factor receptors (such as the HER family receptors) and are known to play important roles in the balance between cell survival and apoptosis. One known fundamental cellular response of the activation of PI3K in vitro and in vivo is the downstream phosphorylation of Akt [10]. Increased intratumoral phosphorylated Akt (P-Akt) has been linked to decreased radiation responsiveness in various malignancies, including head and neck squamous cell carcinoma, lung carcinoma, glioblastoma, and prostate and breast cancer [6,9,10]. Therefore, it has also been postulated that the inhibition of PI3K may provide an additional targeted approach to therapy for this disease process.

We have recently reported that increased expression of HER2 and decreased expression of EGFR membranous staining correlate with improved overall survival in patients with carcinoma of the cervix. We also found that overexpression of HER2 was correlated with adenocarcinoma, and overexpression of EGFR correlated significantly with squamous cell histology [11]. Few studies have evaluated the prognostic utility of multiple HER family biomarkers in carcinoma of the cervix for patients treated in a homogeneous fashion (such as definitive radiotherapy alone). The goals of this study were to evaluate the correlation between members of the HER family and P-Akt in carcinoma of the cervix, and to analyze their prognostic significance in regard to disease-free survival and overall survival by both univariate and multivariate analysis.

Materials and methods

Fifty-five patients who received radiotherapy alone with definitive intent from 1981–1996 on whom tissue blocks were available were included within this study. The patients included within this group were comprised of FIGO stage IB through IVA carcinoma of the cervix. All patients were treated at a single radiation therapy center with standard external beam radiation techniques (with MV photon energies) and the brachytherapy component was delivered with low-dose rate brachytherapy for all patients included in the analysis. The specifics of radiotherapy treatment technique and dose for this patient cohort are described elsewhere [11]. Approval for this study was obtained from the LDS Hospital Institutional Review Board (IRB). Patients who received concurrent chemotherapy or prior surgery for curative intent were excluded from the analysis to provide a more homogenous population for analysis. Medical charts and the hospital registry were also reviewed for clinical parameters and disease status.

The presence of tumor and histopathologic grade was verified by one pathologist prior to performing the specific immunohistochemical analyses. In order to evaluate significant correlations between tumor histology and biomarker expression, patients were divided into squamous cell carcinoma and adenocarcinoma groups (including adenosquamous carcinoma). Immunohistochemistry was performed for EGFR, HER2, HER3, HER4, and P-Akt. The cellular expression of each biomarker was then quantitatively scored by ChromaVision cellular imaging technology for each patient (intensity scale based on pixel density, 0–16000 for EGFR, 0–4.0 for HER2, 0–4.0 for HER3, 0–4.0 for HER4, and 0–16,000 for P-Akt). Overexpression of EGFR, HER2, HER3, HER4, and P-Akt was defined as $\geq$7380, $\geq$2.0, $\geq$0.8, $\geq$2.4, and $\geq$3780 (intensity scale), respectively.

Immunohistochemistry

Formalin-fixed, paraffin-embedded, 5-μm thick sections were prepared from biopsy specimens. Immunohistochemical stains were individually assessed without knowledge of patient outcome. Each slide was first deparaffinized and heated in citrate buffer. After cooling and rinsing in a Tris buffer, immunohistochemical staining was performed with an automated immunohistochemical autostainer.

Detection of EGFR was performed with a secondary mouse anti-immunoglobulin linked to biotin following incubation with streptavidin linked to horseradish peroxidase. The EGFR detection kit was obtained from Dako (M3563) and was used in a concentration of 1:200. Detection of HER2 was performed with a secondary rabbit anti-immunoglobulin linked to biotin following incubation with streptavidin linked to horseradish peroxidase. The HER2 detection kit was obtained from Dako (A0485) and was used in a concentration of 1:200. Detection of HER3 and HER4 was performed with secondary mouse anti-immunoglobulins linked to biotin following incubation with streptavidin linked to horseradish peroxidase. The HER3 and HER4 detection kits were obtained from NeoMarkers (MS-725-P and MS-637-P, respectively) and were used in a concentration of 1:20 and 1:40, respectively. Detection for P-Akt was performed with a secondary rabbit anti-immunoglobulin linked to biotin following incubation with streptavidin linked to horseradish peroxidase. The P-Akt detection kit was obtained from Cell Signaling (9277) and was used in a concentration of 1:50. Color development for all assays was accomplished with diaminobenzidine as the chromagen. Due to the limited quantity of biopsy specimen in select cases, specific immunohistochemically stained slides were unable to be analyzed either because of lack of specimen, an abundance of necrotic tissue, or too little carcinoma to be accurately stained and quantitatively scored by Chromavision software.

Statistics

Parametric (Pearson's correlation) and nonparametric correlations (Spearman's rho) were performed between biomarkers. Univariate and multivariate Cox proportional hazards modeling were performed with disease-free survival (DFS) and overall survival (OS) as the primary endpoints, and each biomarker was evaluated for correlation between various pretreatment clinical factors. The Kaplan–Meier method was utilized to evaluate for differences in OS and DFS for specific prognostic factors. The median time of follow-up was 24 months for all patients and 69 months for living patients. Differences were considered significant when the probability of error was below 5% ($P < 0.05$).

Results

Table 1 lists the pretreatment characteristics of this series of 55 patients with FIGO stage IB through IVA carcinoma of the