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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Low Levels of Phosphorylated Epidermal Growth Factor Receptor in Nonmalignant and Malignant Prostate Tissue Predict Favorable Outcome in Prostate Cancer Patients

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Abstract

Purpose: To explore if the expression of phosphorylated epidermal growth factor receptor (pEGFR) in nonmalignant and malignant prostate tissue is a potential prognostic marker for outcome in prostate cancer patients.

Experimental Design: We used formalin-fixed tissues obtained through the transurethral resection of the prostate from 259 patients diagnosed with prostate cancer after the transurethral resection of the prostate, and patients were then followed with watchful waiting. Tissue microarrays of nonmalignant and malignant prostate tissue were stained with an antibody against pEGFR. The staining pattern was scored and related to clinicopathologic parameters and to outcome.

Results: Low phosphorylation of EGFR in prostate epithelial cells, both in the tumor and surprisingly also in the surrounding nonmalignant tissue, was associated with significantly longer cancer-specific survival in prostate cancer patients. This association remained significant when Gleason score and local tumor stage were added together with pEGFR to a Cox regression model. Tumor epithelial pEGFR immunoreactivity was significantly correlated to tumor cell proliferation, tumor vascular density, and nonmalignant epithelial pEGFR immunoreactivity. Patients with metastases had significantly higher immunoreactivity for tumor and nonmalignant epithelial pEGFR compared with patients without metastases.

Conclusions: Low pEGFR immunoreactivity is associated with the favorable prognosis in prostate cancer patients and may provide information about which patients with Gleason score 6 and 7 tumors that will survive their disease even without treatment. Changes in the nonmalignant tissue adjacent to prostate tumors give prognostic information. *Clin Cancer Res; 16(4); 1245–55. ©2010 AACR.*

Some prostate cancer patients have a rapidly progressing lethal disease, but the majority of patients have long expected survival (1). Although curative treatments may reduce the risk of progression and prostate cancer mortality, these treatments have adverse effects including erectile dysfunction and incontinence (2), and a large proportion of patients would have survived their prostate cancer even without treatment (3, 4). There are several prognostic tools available for clinical management of prostate cancer (5), and the Gleason score (GS) system (6) is the strongest predictor of outcome, especially for low (≤5) and high (8–10) GS tumors (2, 3). Currently >70% of the localized tumors are graded as GS 6 or 7 on diagnostic biopsies and these patients have highly variable and largely unpredictable outcome (2). Furthermore, at present, there is no imaging method available that detects prostate cancer, and therefore, diagnostic biopsies often sample only nonmalignant prostatic tissue (7). Biopsies that contain prostate tumors are often undergraded as only a small proportion of the prostate gland is sampled. Therefore, additional prognostic markers are urgently needed. Such markers should ideally be detectable also in the nonmalignant prostate tissue and indicate the presence and aggressiveness of tumors elsewhere in the organ.

Prostate growth and function are controlled by locally secreted autocrine and paracrine regulators. One of these regulatory systems is the epidermal growth factor receptor (EGFR) and its ligands (amphiregulin, βcellulin, epidermal growth factor, heparin-binding epidermal growth

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

We propose that phosphorylated epidermal growth factor receptor (pEGFR) immunoreactivity when validated in other patient cohorts, particularly in current diagnostic biopsies from prostate-specific antigen–detected cases, could possibly become a useful prognostic marker for prostate cancer. The immunoreactivity of pEGFR may provide information about which patients with Gleason score 6 and 7 tumors will survive their disease even without treatment. Changes of pEGFR immunoreactivity in the nonmalignant prostate tissue adjacent to prostate tumors give prognostic information and may possibly be used as a novel diagnostic method. Staining for pEGFR could then possibly, when validated in other studies, be used to classify patients with negative biopsies according to their need for close follow-up. Staining for pEGFR could also possibly be used to select patients that may benefit from anti-EGFR treatment.

factor, and transforming growth factor α; refs. 8–11). The EGFR is a tyrosine (TYR) kinase receptor, expressed in normal tissues in many organs and in many different types of tumors (12), and part of a subfamily of four closely related receptors: EGFR (HER-1, ErbB-1), HER-2 (ErbB-2/neu), HER-3 (ErbB-3), and HER-4 (ErbB-4; ref. 13). Increased expressions of these receptors have all been described in prostate cancer (14, 15). The EGFR forms homodimeric or heterodimeric complexes (usually with HER-2) when bound to its ligands. This leads to the phosphorylation of nine different TYR residues, through either receptor TYR kinase autophosphorylation or through Src-dependent phosphorylation (16, 17). The activation of EGFR results in various responses including proliferation, differentiation, secretion, migration, angiogenesis, and inhibition of apoptosis (9, 12). Aberrant EGFR signaling is involved in tumor progression and metastases in several human cancers (18). In the prostate, EGFR is expressed mainly in epithelial cells (11). Increased levels of EGFR in prostate tumor epithelial cells from radical prostatectomy specimens have been correlated to high GS and early prostate-specific antigen (PSA) relapse. Activation of EGFR signaling also plays a critical role during progression to castration-resistant and metastatic prostate cancer (5, 22–25). Anti-EGFR treatment prevents the growth of castration-resistant prostate cancer (26, 27). We have previously shown that castration-induced prostate involution and testosterone-stimulated normal prostate growth could be inhibited by anti-EGFR treatment (8). Thus, EGFR plays a major role in androgen-regulated prostate growth and regression (8).

In this study, we wanted to elucidate the role of phosphorylated EGFR (pEGFR) as a potential prognostic biomarker for prostate cancer. We therefore studied whether the immunoreactivity of pEGFR on TYR residue 845 (TYR845) is related to the outcome in a historical set of Swedish men diagnosed with prostate cancer after the transurethral resection of the prostate (TURP) and who were then managed with watchful waiting and an extremely long follow-up. In contrast to contemporary PSA-detected and prostatectomized prostate cancer cases, such patients are one of the few possibilities currently available (28, 29) to identify potential novel biomarkers for patients that will not require aggressive treatment.

Materials and Methods

Patients. Between 1975 and 1991, tissue specimens were obtained from patients who underwent TURP at the hospital in Västerås, Sweden, due to obstructive voiding problems. Subsequent histologic analysis showed the presence of prostate cancer. At that time, serum PSA was not yet used for diagnostics in Sweden. Median age at TURP was 74 y (range, 51-95 y). Tissue specimens were formalin-fixed, paraffin-embedded, and regraded according to the Gleason system (3). Radionuclide bone scan was done shortly after diagnosis for the detection of metastases. Patients had not received any anticancer therapy before TURP. The study includes 303 patients, of which 259 patients were followed with watchful waiting after TURP. At the onset of symptoms from metastases, patients received palliative treatment with androgen ablation. In a few cases, patients received radiation therapy or estrogen therapy, according to therapy traditions in Sweden during that time period. In addition, we analyzed 44 patients that were treated with palliative treatment immediately after diagnosis. The median overall survival was 5.4 y. In TURP specimens, 55 patients had tumors graded as GS 4 to 5; 98 patients had tumors graded as GS 6; 60 patients had tumors graded as GS 7; and 90 patients tumors graded as GS 8 to 10. One patient (1%) with GS 6, 3 patients (5%) with GS 7, and 25 patients (28%) with GS 8 to 10 had bone metastases at diagnosis. The cause of death was assessed by the evaluation of medical records. In August 2003, 32 patients (11%) were still alive, 98 patients (32%) had died from prostate cancer, and 173 patients (57%) had died from other causes. Cancer-specific and relative survival was almost similar, indicating a correct evaluation of the cause of death (3, 30). From the tissue specimens collected, we constructed tissue microarrays (TMA) using a Beecher Instrument. The TMAs contained five to eight samples of tumor tissue representing both the primary and secondary Gleason grade (cores with a diameter of 0.6 mm), and four samples of nonmalignant tissue from each patient (31, 32). The local Human Investigations Committee has approved this study.

Immunohistochemistry. Four-micrometer sections were deparaffinated, rehydrated, washed, and microwave heated in citrate buffer (pH 6.0). Immunohistochemistry staining was done using a primary polyclonal rabbit antibody against pEGFR (TYR845 lot 4, Cell Signaling; 1:50) and enhanced by a catalyzed signal amplification system (DAKO Corp.). Others have previously used this antibody

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in Western blot and immunohistochemistry to detect pEGFR on TYR845 (33–35). Slides were incubated with hydrogen peroxidise to quench the endogenous peroxidise activity. Sections were incubated with protein block solution before they were incubated with the primary pEGFR antibody overnight followed by incubations with biotinylated secondary antibody. Staining was completed when sections were incubated with diaminobenzadine tetrahydrochloride and counterstained with Mayer's hematoxylin. Staining for the von Willebrand factor (vWf/factor VIII–related antigen, DAKO) and Ki-67 (DAKO) was done as previously described (36). Control sections that were included were primary pEGFR antibody that had been preincubated with excess of corresponding peptide, or with phosphatase in which sections were incubated at 37°C with 3 μL phosphatase enzyme solution in a volume of 100 μL NEObuffer containing $MnCl_2$ (New England Biolabs) or the control solution without phosphatase enzyme for 2 h, followed by immunostaining as described above. For comparison, we also stained consecutive TMAs with pEGFR TYR845 lot 7, pEGFR TYR1068, and pEGFR TYR1173 (Cell Signaling) as described above. We also immunostained consecutive TMAs with antibodies against EGFR (M7239, DAKO) using Ventana DAB iView system and against human EGFR-2 (HER2; c-erbB2 Cocktail, Biocare Medical) using ABC reagent (Vector laboratories) with 3,3′-diaminobenzidine (DAKO) as chromogen.

Scoring of pEGFR staining. The immunoreactivity was evaluated by two different investigators (P. H., 303 patients and A. K., 166 patients) with no knowledge of the patient data, and similar results were obtained in the survival analysis (data not shown).

To quantify the pEGFR immunostaining, a score combining the staining intensity and distribution was used. Tumor epithelial cells, luminal epithelial cells, and basal epithelial cells were quantified separately. Staining intensity and the distribution for pEGFR was scored as 0 (no staining), 1 (predominantly unstained with smaller stained areas), 2 (stained and unstained areas are about equally large), 3 (predominantly stained tissue with smaller unstained areas), 4 (all epithelial cells are moderately stained), and 5 (all epithelial cells are strongly stained). Thus, tissue specimens scored from 0 to 3 were scored according to the distribution of the stained areas and not to intensity. This scoring was used because the varieties in staining intensity were rather small between the stained areas in each tissue specimen scored from 0 to 3, i.e., they showed staining of low intensity. In tissue specimens scored 4 or 5, all the cells were stained but differences were observed in intensity, low or high. The pEGFR staining score are the mean values of five to eight scored samples of tumor tissue or four scored samples of nonmalignant tissue.

Tumor size, local tumor stage, microvessel density, and Ki-67 labeling index. Data on tumor size, local tumor stage, microvessel density, and Ki-67 labeling index have previously been quantified for these tumors (3, 36), and these data were related to pEGFR levels.

Statistics. Bivariate correlations were calculated using the Spearman's rank correlation test. Correlations between nominal variables and continuous variables were analyzed using the Kendall's τ b correlation test. Data used in the correlation analysis were collected at the time of prostate cancer diagnosis.

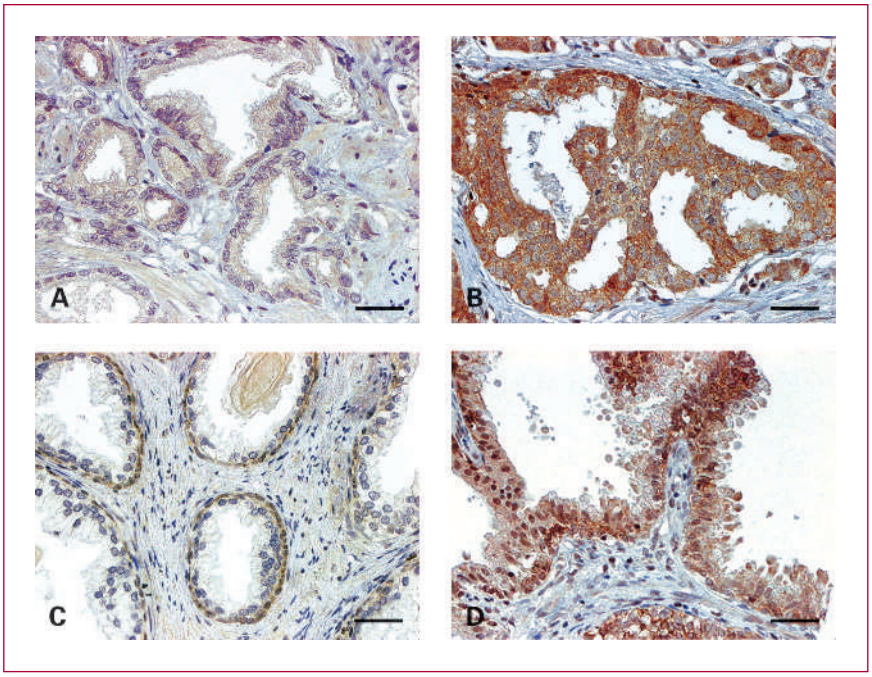

Fig. 1. Prostate tissue immunostained for pEGFR TYR 845. Bars, 20 μm. A, largely unstained tumor tissue. B, tumor tissue with high immunoreactivity. C, nonmalignant tissue with moderate immunoreactivity in basal epithelial cells. D, nonmalignant tissue with high immunoreactivity in basal and luminal epithelial cells.

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Patients included in the survival analyses with the Kaplan-Meier and Cox regression were followed with watchful waiting, thereby allowing for an evaluation of pEGFR as a prognostic marker upon the natural progression of the disease. The duration of event-free survival (EFS) is defined as the time from TURP until the date of prostate cancer death, death of other causes, or if no death occurred, until the date of last follow-up. Differences in outcome between groups were tested with the log-rank test. The prognostic relevance of pEGFR immunoreactivity was examined by Cox regression analysis alone and together with GS and local tumor stage.

Groups were compared with the Mann-Whitney *U* test. Means and medians (±SD), and probability of EFS (P-EFS; ±SEM) are presented. The level of statistical significance was defined as $P < 0.05$ (two sided). Statistical analysis was done using the SPSS 14.0.0 software for Windows (SPSS, Inc.).

Results

Immunoreactivity for pEGFR TYR845. Tumor epithelial cells showed cytoplasmic, membrane, and nuclear immunoreactivity for pEGFR TYR845 (Fig. 1A-B). In nonmalignant basal epithelial cells with low to moderate cytoplasmatic, membrane, and nuclear pEGFR immunoreactivity, the luminal epithelial cells were generally unstained (Fig. 1C). Conversely, in those in which basal epithelial cell staining was particularly strong, the luminal epithelial cells showed pEGFR staining (Fig. 1D). Increased expression in one cellular compartment was associated with increased expression in the others. Tumor and epithelial cells had varying staining intensity and distribution in different patients, whereas stroma and vascular cells were largely unstained. Within individual patients, the staining variability for tumor, nonmalignant luminal, and basal epithelial pEGFR was low.

Specificity of the pEGFR TYR845 staining. Incubating sections with an excess of antigen (Supplementary Fig. S1) or phosphatase (Supplementary Fig. S2) repressed the pEGFR staining in all cell compartments including the nuclei. In addition, we have previously seen that staining with this particular antibody is ablated by treatment with an EGFR TYR kinase inhibitor *in vivo* (8), collectively suggesting that the staining is pEGFR specific. Sections stained with another batch of antibody against pEGFR TYR845 (lot7) and antibodies against other phosphorylation sites of the pEGFR, TYR1173, and TYR1068 showed very similar staining patterns as pEGFR TYR845 (lot4). The staining scores in the luminal epithelial cells in nonmalignant glands for these antibodies were highly correlated (R_S between 0.82 and 1.0; $P < 0.004$; $n = 10$; for all combinations; Supplementary Table S1). When comparing sections stained with an EGFR antibody (detects both the phosphorylated and unphosphorylated receptor) and the pEGFR TYR845 (lot4) antibody, it was noted that both antibodies stained the same cell types and pEGFR was not detected in EGFR-negative cells (Supplementary Fig. S3). Notably, cells with strong EGFR staining did not always show strong pEGFR staining, suggesting that EGFR is not always phosphorylated. As the antibody for pEGFR, according to the manufacturer, may cross-react with HER2, we compared sections stained for pEGFR and HER2. In nonmalignant glands, HER2 staining was observed in the basal layer and sometimes in luminal epithelial cells, but generally not in the nucleus (Supplementary Fig. S3). Tumor cells showed HER2 staining with varying intensity, but HER2 and pEGFR staining were not identical (Supplementary Fig. S4). This comparison between HER2 and pEGFR suggests that these two antibodies stain at least partly different antigens.

Correlation analysis and comparison of means. The pEGFR TYR845 scores in prostate tumor epithelial cells and in nonmalignant epithelial cells correlated with each other and with GS, metastasis, tumor size, local tumor stage, and tumor cell proliferation (Table 1). Tumor pEGFR also correlated with tumor vascular density (Table 1). Patients with metastases had significantly higher pEGFR in tumor cells and in nonmalignant luminal and basal epithelial cells compared with patients without metastases (3.67 ± 0.60 versus 3.05 ± 0.96, $P < 0.001$, $n = 226$; 3.56 ± 0.82 versus 2.78 ± 1.09, $P < 0.001$, $n = 220$; 3.82 ± 0.56 versus 3.26 ± 0.91, $P = 0.004$, $n = 220$, respectively).

Receiver operating curve analysis, test set, validation set, and cutoffs. The cutoffs for pEGFR TYR845 was obtained in a test set (two of three of the patients on watchful waiting) on the basis of the receiver operating curve analysis. Receiver operating curve analysis showed areas under the curve of 0.63 and 0.63 for staining in tumor cells and nonmalignant luminal cells, respectively, in the test set (95% confidence interval, 0.54-0.72 and 0.54-0.72). Cutoffs used in survival analysis was 2.78 and 2.88 for tumor and nonmalignant luminal epithelial cell staining, i.e., high pEGFR immunoreactivity was ≥2.78 for tumor and ≥2.88 for nonmalignant luminal cells. Cutoffs obtained were used in the Kaplan-Meier analysis in a test set and in a validation set (one of three of the patients on watchful waiting; Fig. 2). These survival analyses showed that low pEGFR immunoreactivity in prostate epithelial cells, both in tumor cells and in surrounding nonmalignant luminal epithelial cells, were associated with significantly longer cancer-specific survival (Fig. 2).

The whole patient material ($n = 259$) was then used in the correlation analysis and in the more detailed survival analyses with the Kaplan-Meier and Cox regression. Further analyses of the whole watchful waiting material showed that cutoffs could also be set to the mean, median, or to 3, obtaining similar results in the Kaplan-Meier and Cox regression analysis (data not shown).

Tumor pEGFR immunoreactivity and clinical outcome. Patients with high tumor pEGFR TYR845 had significantly shorter cancer-specific survival than patients with low tumor pEGFR TYR845 (15-year P-EFS was 48 ± 5% and 84 ± 5% in the two groups; Fig. 3). In addition, high tumor pEGFR in patients with a GS 6 or 7 (15-year P-EFS, 50 ± 8%) and in patients with a GS 6 (15-year P-EFS, 57 ± 11%) had significantly shorter cancer-specific survival than

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those with low tumor pEGFR in these subgroups (15-year P-EFS was 87 ± 6% and P-EFS was 97 ± 3%, respectively; Fig. 3). High tumor pEGFR was associated with an increased relative risk for prostate cancer–specific death in a univariate Cox regression analysis (Table 2A). In the multivariate Cox regression analysis including the known prognostic marker GS and local tumor stage, high tumor pEGFR was significantly associated with poor prognosis and was an independent prognostic marker (Table 2B).

Nonmalignant pEGFR immunoreactivity and clinical outcome. Patients with high nonmalignant luminal pEGFR TY845 had significantly shorter cancer-specific survival than patients with low nonmalignant luminal pEGFR TYR845 (15-year P-EFS was 48 ± 6% and 80 ± 5% in the two groups; Fig. 3). In addition, high nonmalignant luminal epithelial pEGFR in patients with GS 6 or 7 (15-year P-EFS, 53 ± 9%) and in patients with GS 6 (15-year P-EFS, 63 ± 11%) had significantly shorter cancer-specific survival than patients with low nonmalignant luminal pEGFR in the subgroups (15-year P-EFS of 77 ± 9% and P-EFS of 87 ± 9%, respectively; Fig. 3). Patients with high nonmalignant basal pEGFR immunoreactivity also had significantly shorter cancer-specific survival than patients with low nonmalignant basal pEGFR (data not shown). High nonmalignant luminal pEGFR was associated with an increased relative risk for prostate cancer–specific death in a univariate Cox regression analysis (Table 2A). In accordance with tumor pEGFR, nonmalignant luminal pEGFR score was an independent prognostic marker from GS and local tumor stage in multivariate Cox regression analysis (Table 2C).

Tumor and nonmalignant luminal pEGFR immunoreactivity and clinical outcome. A combined variable between tumor and nonmalignant luminal pEGFR TYR845 was constructed and analyzed by Cox regression (Fig. 4). Patients with high tumor pEGFR and high nonmalignant luminal pEGFR had a 3-fold excess risk of prostate cancer death compared with the added separate relative risks for patients identified as having high tumor pEGFR or high nonmalignant luminal pEGFR, respectively (interaction analysis $P < 0.001$).

Discussion

High tumor EGFR immunoreactivity (irrespective of phosphorylation status) has been associated with high

Table 1. Bivariate correlations

		Tumor pEGFR immunoreactivity	Nonmalignant luminal pEGFR immunoreactivity	Nonmalignant basal pEGFR immunoreactivity
Nonmalignant luminal pEGFR*	r	0.51†		0.84†
	n	293		
Nonmalignant basal pEGFR*	r	0.49†	0.84†	
	n	293		
GS*	r	0.30†	0.29†	0.25†
	n	293	293	
Metastases‡	r	0.21†	0.20†	0.17†
	n	220	220	
Local tumor stage*	r	0.18†	0.22†	0.23†
	n	288	288	
Tumor size*	r	0.15§	0.23†	0.19†
	n	293	293	
Tumor cell proliferation* (Ki-67 labeling index)	r	0.27†	0.26†	0.25†
	n	284	284	
Nonmalignant epithelial cell proliferation* (K-i67 labeling index)	r	–0.01	–0.002	0.02
	n	287		
Tumor vascular density* (vWf-stained vessels)	r	0.21§	0.11	0.08
	n	152		
Nonmalignant vascular density* (vWf-stained vessels)	r	0.14	0.07	0.14
	n			

NOTE: Data used in the correlation analysis were collected at the time of prostate cancer diagnosis.
Abbreviation: vWf, von Willebrand factor.
*Spearman's rank correlation test.
†Correlation is significant at the <0.005 level (two tailed).
‡Kendall's τ b correlation test.
§Correlation is significant at the <0.05 level (two tailed).

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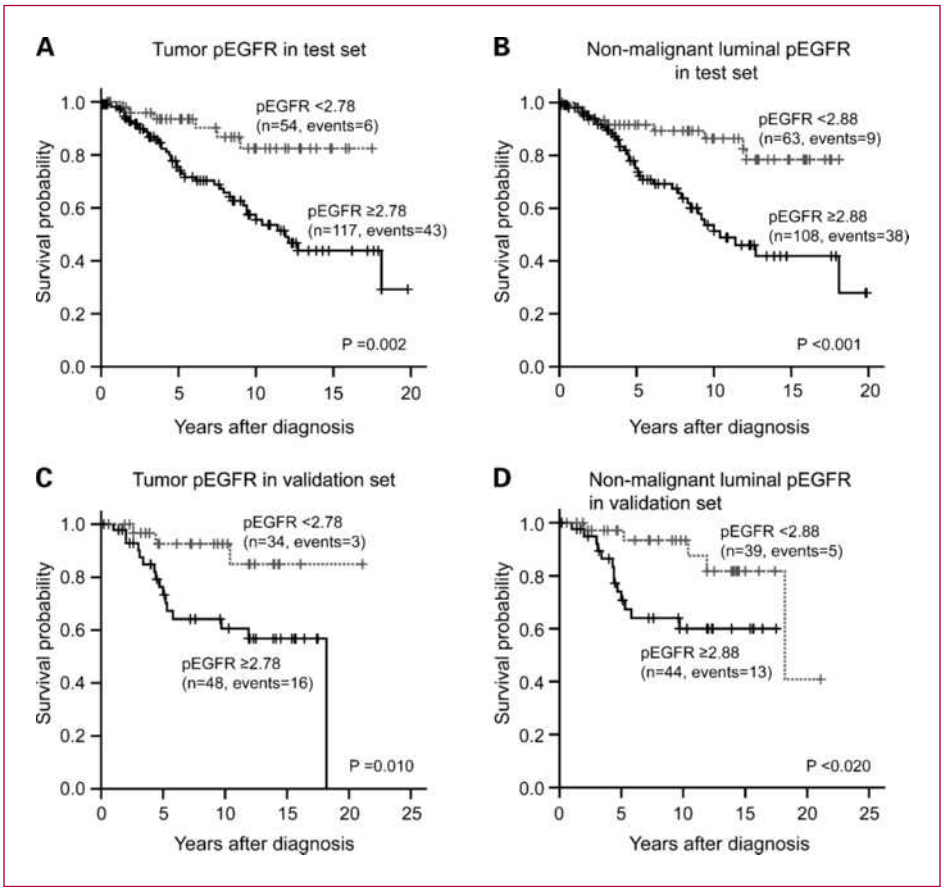

Fig. 2. Survival curves. Patients divided into two groups depending on immunoreactivity of tumor pEGFR TY845 or of nonmalignant luminal pEGFR TYR845 in the test set (A and B) and in the validation set (C and D). Solid line, high tumor pEGFR (≥2.78); dashed line, low tumor pEGFR (<2.78; A and C). Solid line, high nonmalignant luminal pEGFR (≥2.88); dashed line, low nonmalignant luminal pEGFR (<2.88; B and D).

Gleason grade, advanced tumor stage, and high risk for PSA recurrence (19–23). The percentage of cases showing increased EGFR staining was however highly variable (1-100%) and EGFR staining was not always an independent prognostic marker (19). To our knowledge, pEGFR expression has previously not been examined in prostate cancer, but studies in non–small cell lung cancer and breast cancer patients have indicated that high expression in the tumor is associated with poor outcome (35, 37, 38). Low immunoreactivity for pEGFR at TYR845 in prostate epithelial cells, both in the tumor and in the surrounding nonmalignant glands, was significantly associated with longer cancer-specific survival for prostate cancer patients. As cutoffs, we used scoring values slightly below 3 obtained from the receiver operating curve analysis in a test set. Cutoffs were then verified in survival analysis in both the test set and a validation set. The cutoff could however also be set to 3 with a similar ability to separate cases with different prognosis. This means that prostate cancer patients that have tumor or nonmalignant luminal epithelial tissue in which most of or all the cells are immunoreactive for pEGFR have poor survival. This phenotype can easily be scored in the microscope, making it a potentially practically useful marker. pEGFR immunoreactivity could independently predict prostate cancer outcome in a subgroup of patients with GS 6 to 7 tumors and in a subgroup of patients with GS 6 tumors. We therefore propose that the quantification of pEGFR immunoreactivity (by manual scoring or preferably by image analysis) when validated in other patient cohorts, particularly in current diagnostic biopsies from PSA-detected cases, could possible become a prognostic marker for prostate cancer.

To use pEGFR staining as a prognostic marker, the antibody must be specific and the staining protocol must be robust. Even if the antibody used in this study is not ideal, it seems to be fairly specific and the staining score for pEGFR TYR845 (lot4) correlated with other pEGFR antibodies. In addition, it was ablated by treatment with an EGFR TYR kinase inhibitor (8). The pEGFR antibody for TYR845 that was used in this study may, however, cross-react with HER-2. HER2 and pEGFR staining were not identical and HER2 was a weaker prognostic biomarker than pEGFR in tumor tissue. HER2 also did not work at all in nonmalignant tissue.[5] Our findings with pEGFR can therefore not be fully explained by

[5] P. Hammarsten, unpublished observations.

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cross-reaction with HER-2. EGFR is generally considered to be located in the cell membrane, but in addition to staining in cell membranes and cytoplasm, pEGFR immunoreactivity was also located in the nucleus. The nuclear staining is, however, apparently not unspecific, and after ligand stimulation, EGFR moves to the nucleus and functions as a transcriptional regulator activating for example cyclin D1 (39). In addition, nuclear pEGFR has recently been shown to be a prognostic marker in other types of tumors (40). Cyclin D1 plays a major role in prostate tumorigenesis and promotes bone metastases (41). Phosphorylation of EGFR at TYR845 (and TYR1173, which is highly correlated with TYR845) is mediated by c-Src and is involved in the regulation of the receptor function, as well as in prostate tumor progression (16, 35). C-Src is a nonreceptor protein kinase responsible for signal

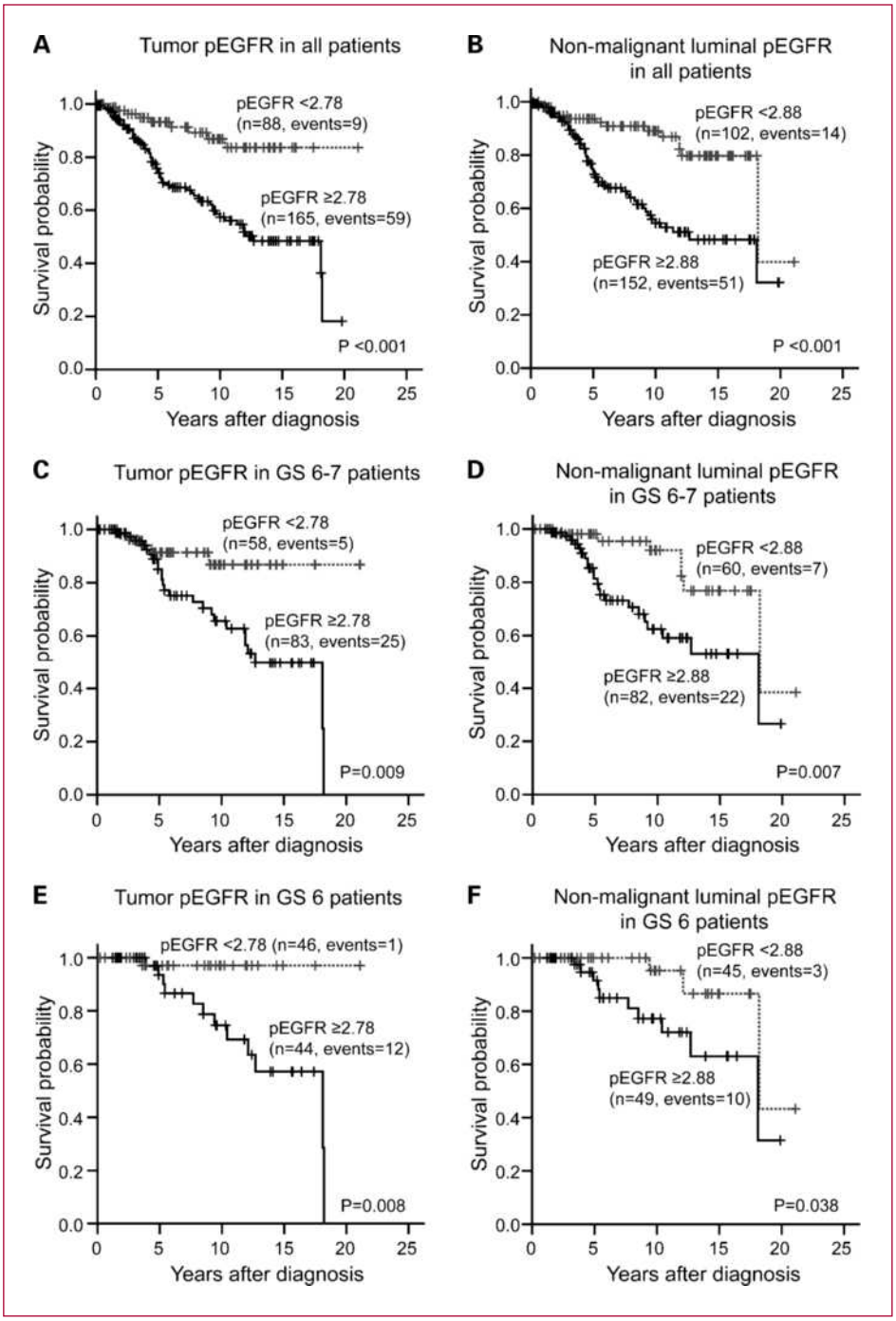

Fig. 3. Survival curves. Patients divided into two groups depending on the immunoreactivity of tumor pEGFR TYR845 or of nonmalignant luminal pEGFR TYR845 in all patients (A and B), patients with GS 6 or 7 (C and D), and patients with GS 6 (E and F). Solid line, high tumor pEGFR (≥2.78); dashed line, low tumor pEGFR (<2.78; A, C, E). Solid line, high nonmalignant luminal pEGFR (≥2.88); dashed line, low nonmalignant luminal pEGFR (<2.88; B, D, F).

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transduction during many cellular activities, including differentiation, adhesion, and migration (42). Aberrant c-Src activity promotes prostate cancer progression and androgen-independent growth (42). Staining for pEGFR may therefore possibly be used to select patients that could benefit from anti-EGFR or anti-Src treatments.

The most intriguing finding in this article is that changes in the nonmalignant prostate tissue can perhaps be used to predict the presence and aggressiveness of prostate cancer elsewhere in the organ. Surprisingly, only few studies have explored the possibility that the nonmalignant tissue adjacent to prostate tumors express prognostic markers (7, 43, 44). Changes in the nonmalignant prostate tissue adjacent to prostate cancer, detected by altered magnetic resonance spectra, however, provide prognostic information (45). Increased phosphorylated activated protein kinase immunoreactivity in nonmalignant epithelial cells adjacent to prostate tumors independently predict biochemical recurrence in patients with GS 6 or 7 tumors (43, 44). As activation of EGFR results in the phosphorylation of AKT (46), it is possible that the increased phosphorylated activated protein kinase immunoreactivity could be caused by increased EGFR signaling. We have shown in the patient cohort used in this study that decreased androgen receptor levels in the stroma in the nonmalignant parts of the prostate was associated to poor outcome (47). In addition, in diagnostic biopsies, low androgen receptor immunorectivity in the stroma

Table 2. Cox regression for tumor and nonmalignant luminal epithelial pEGFR of patients followed with watchful waiting

Variable		*n*	RR	*P*	95% confidence interval
A. Univariate analysis					
GS*	4-5	54	1†		
	6-7	145	17.7	0.005	2.4-129.9
	8-10	60	93.2	<0.001	12.7-683.5
Local tumor stage*	T1a-T1b	160	1†		
	T2	62	4.1	<0.001	2.3-7.4
	T3	32	8.9	<0.001	4.7-16.8
	T4	3	10.6	0.022	1.4-81.0
Tumor pEGFR*	<2.78	88	1†		
	≥2.78	165	3.9	<0.001	1.9-7.9
Nonmalignant luminal pEGFR*	<2.88	102	1†		
	≥2.88	152	3.3	<0.001	1.8-6.0
B. Multivariate analysis					
GS*	4-5	53	1†		
	6-7	141	16.0	0.007	2.2-117.8
	8-10	57	50.5	<0.001	6.6-386.0
Local tumor stage*	T1a-T1b	155	1†		
	T2	61	2.1	0.021	1.1-3.8
	T3	32	2.4	0.021	1.1-4.9
	T4	3	4.1	0.175	0.5-31.8
Tumor pEGFR*	<2.78	87	1†		
	≥2.78	164	2.3	0.027	1.1-4.6
C. Multivariate analysis					
GS*	4-5	54	1†		
	6-7	142	14.0	0.010	1.9-103.1
	8-10	56	40.4	<0.001	5.3-310.0
Local tumor stage*	T1a-T1b	158	1†		
	T2	62	2.3	0.008	1.2-4.4
	T3	29	2.8	0.008	1.3-6.1
	T4	3	4.6	0.144	0.6-35.7
Nonmalignant luminal pEGFR*	<2.88	102	1†		
	≥2.88	150	2.1	0.022	1.1-3.8

Abbreviations: RR, relative risk.

*Cox regression analysis using GS, tumor, and nonmalignant luminal pEGFR as categorical variables.

†Reference value.

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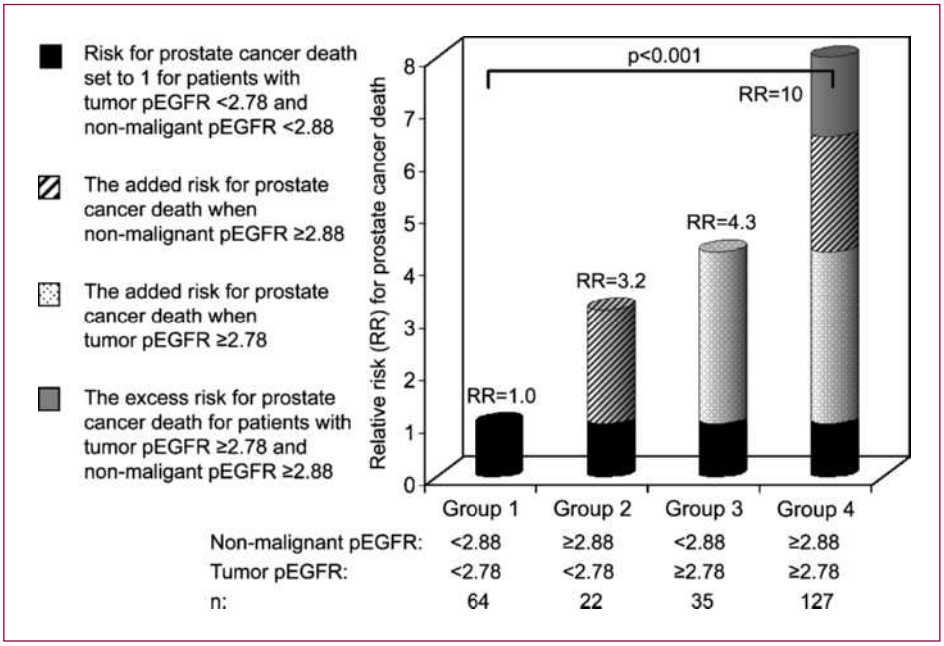

Fig. 4. The diagram shows the interaction with regard to outcome between nonmalignant and tumor pEGFR TYR845 immunoreactivity. It also illustrates the excess/interaction effect of high nonmalignant pEGFR and high tumor pEGFR of patients followed with watchful waiting.

of the nonmalignant compartment was associated with a poor response to castration therapy (47). Defining changes in the nonmalignant tissue surrounding a tumor could thus be of diagnostic importance, as diagnostic biopsies often sample only nonmalignant prostatic tissue also when tumors are present (7). Staining for pEGFR and other changes such as the androgen receptor (47) could possibly, when validated in other studies, be used to classify patients with negative biopsies according to their need for close follow-up.

The mechanisms behind increased EGFR phosphorylation in prostate tumors and particularly in adjacent nonmalignant tissue also remain to be clarified. Some patients may have an inherent predisposition to express high level of pEGFR and may therefore have an adverse prognosis. It is also possible and perhaps more likely that the tumor secretes EGFR ligands or other factors causing high pEGFR in the surrounding tissue. In experimental animals, tumors alter the morphology of the surrounding nonmalignant prostate tissue and the magnitude of this is probably related to the distance to the tumor (48). pEGFR levels in the nonmalignant tissue are significantly associated with tumor size (present study), and factors such as PTEN, p27, and Ki-67 are in patients altered in normal glands close to prostate tumors (44).

One likely reason to the increased pEGFR immunoreactivity and other changes in the nonmalignant tissue adjacent to tumors could thus be that tumors influence their surroundings. The nonmalignant tissue adjacent to prostate tumors should therefore not be classified as normal (7, 43, 44). To stimulate further studies and discussion, we have proposed that the nonmalignant tissue adjacent to prostate tumors could be called "tumor indicating nonmalignant tissue" (TINT; ref. 47). Further studies are obviously needed to explore how prostate tissue could be "tinted" by the presence of a tumor and the possible use of this in prostate cancer diagnosis. Increased pEGFR immunoreactivity in the nonmalignant and tumor compartment was associated with an excess risk, compared with patients in which pEGFR only was increased in the tumor. Tumor indicating nonmalignant tissue changes, such as increased luminal epithelial pEGFR immunoreactivity, decreased stroma androgen receptor immunorectivity (47), and increased blood supply through the surrounding nonmalignant tissue (48, 49), may thus influence outcome and, when more closely defined, be potential new targets for therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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