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Epidermal Growth Factor Receptor Overexpression is a Marker for Adverse Pathologic Features in Papillary Thyroid Carcinomas

Kevin E. Fisher, M.D., Ph.D.¹, Jigna C. Jani, M.D.¹, Sarah B. Fisher, M.D.², Cora Foulks, M.S.¹, Charles E. Hill, M.D., Ph.D.¹, Collin J. Weber, M.D.², Cynthia Cohen, M.D.¹, and Jyotirmay Sharma, M.D.²

¹Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

²Department of Surgery, Emory University School of Medicine, Atlanta, GA

Abstract

Background—Epidermal growth factor receptor (EGFR) overexpression (EGFR-H) is implicated in thyroid carcinoma disease progression, but the clinicopathologic significance of EGFR-H in tumors that harbor *EGFR* and/or *BRAF(V600E)* mutations is unknown.

Methods—Tissue microarrays from 81 patients who underwent thyroidectomies for carcinoma from 2002–2011 were scored for EGFR expression using immunohistochemistry (IHC). Somatic mutations in *EGFR* exons 19 and 21 and *BRAF* were analyzed. Correlations between EGFR IHC, *EGFR*, and *BRAF(V600E)* mutations and clinicopathologic features were assessed.

Results—EGFR-H was detected in 39.5% of carcinomas (n=32) from patients with papillary (PTC, 46.2%, n=18), follicular (29.6%, n=8), and anaplastic (ATC, 100.0%, n=6), but not medullary (0.0%, n=9) thyroid carcinoma. *BRAF(V600E)* mutations were identified in 22.2% of carcinomas (n=18, 15 PTCs and 3 ATCs). No somatic *EGFR* mutations were detected in any subtype. On PTC univariate analysis, EGFR-H correlated with increasing stage, extrathyroidal extension (ETE), tumor capsule invasion (TCI), adverse pathologic features (APF: any demonstration of ETE, TCI, lymph-vascular invasion, lymph node metastases, and/or distant metastases), and *BRAF(V600E)* mutations. On multivariate analysis, EGFR-H correlated with *BRAF(V600E)* mutations. In *BRAF* wild-type (*BRAF*-WT) PTCs, the correlation between EGFR-H and APF approached statistical significance (p=0.065).

Conclusions—EGFR-H may be an important biomarker for aggressive PTCs, particularly in *BRAF*-WT PTCs. Despite EGFR-H in PTC, FTC, and ATC by immunohistochemistry, somatic *EGFR* mutations are absent. Therefore, future investigations of EGFR should consider histologic and immunohistochemical methods in addition to molecular profiling of thyroid carcinomas. This multimodality approach is particularly important for future clinical trials testing anti-EGFR therapy.

Keywords

Papillary; Thyroid; Carcinoma; EGFR; BRAF; Molecular; Immunohistochemistry

Introduction

Thyroid carcinoma comprises 3% of all newly-diagnosed human malignancies (5% in women), and accounts for more than 90% of all endocrine cancers (1). The incidence of thyroid carcinoma is rising and is partially attributable to more sensitive diagnostic modalities (2). The prognosis of thyroid cancer depends on several well established clinicopathologic criteria including age, gender, histologic subtype, tumor size, extrathyroidal extension (ETE), and the presence of lymph node (LNM) or distant metastases (3). Recently however, there has been an increase in thyroid carcinoma-related mortality, particularly in men, after a 20-year period of a relatively flat mortality rate (4). Thus, a better understanding of tumor-specific prognostic markers may identify specific subsets of patients who are at risk for adverse outcomes.

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase (TK) receptor that is expressed in a wide variety of neoplastic and non-neoplastic tissues. Constitutively activated EGFR promotes cell proliferation and inhibits apoptotic mechanisms through the mitogen-activated protein kinase (MAPK) signaling pathway. Recently, Landriscina *et al.* demonstrated that papillary thyroid carcinomas (PTCs) express EGFR, and PTCs overexpress EGFR during dedifferentiation or anaplastic transformation (5). EGFR overexpression (EGFR-H) is described in anaplastic thyroid carcinomas (ATCs) (6), follicular thyroid carcinomas (FTCs) (7, 8) and in primary medullary carcinomas (MTC), despite the absence of identifiable somatic *EGFR* mutations (9, 10).

In other malignancies, growth factor receptor dysregulation influences disease progression and response to targeted therapy (11). Specific activating mutations that affect the EGFR TK domain are best characterized in lung adenocarcinoma. The most common are deletions in exon 19 (del 2235-2249/2236-2250; del E746-A750), followed by a point mutation in exon 21 (T>G 2573) which results in substitution of leucine by arginine at codon 858 (L858R) (12). Similar somatic mutations have been described in thyroid cancers (13), but these mutations are not well characterized (14) and some studies fail to identify them at all (10, 15).

v-Raf murine sarcoma viral oncogene homolog B1 (BRAF), a serine/threonine-specific protein kinase, is a member of the MAPK signaling pathway and a downstream effector of EGFR signaling (16). The *BRAF(V600E)* (T>A 1799) point mutation results in the substitution of valine with glutamate in codon 600 and converts BRAF into a dominant transforming protein that causes constitutive activation of the MAPK pathway. This mutation is present in numerous cancers but occurs with increased frequency in PTC and is considered an independent oncogenic event for PTC tumorigenesis (17). The *BRAF(V600E)* mutation has been reported in PTC and ATC, but not in FTC, MTC, or benign thyroid neoplasms (18).

Extensive research has focused on *BRAF(V600E)* status as an independent marker of adverse outcome (19-21). However, *BRAF(V600E)* is not a sufficient marker for an aggressive phenotype as many *BRAF* wild-type (*BRAF*-WT) thyroid carcinomas exhibit aggressive behavior (22). In one series, 25% of *BRAF*-WT thyroid carcinomas demonstrated ETE, LNM, and disease recurrence (23). Additionally, a recent report using high-throughput proteomic analysis showed that when PTCs were stratified by morphologic classification, *BRAF* mutations did not predict invasive behavior (24). Collectively, these data highlight the need for additional prognostic markers, particularly in *BRAF*-WT carcinomas.

It is clear that the pathogenesis and biologic behavior of thyroid carcinoma involves aberrant MAPK signaling, and EGFR and BRAF are principally dysregulated in PTC and ATC. Though recent evidence implicates EGFR-H in advanced thyroid carcinoma progression (5), the clinicopathologic significance of *EGFR* or *BRAF(V600E)* mutations in thyroid carcinomas with EGFR-H is poorly understood. A better understanding of the molecular mutational status in addition to IHC expression is required for effective therapeutic modalities targeting EGFR (14).

In this study we investigated the utility of EGFR-H as assessed by IHC and molecular mutational analysis as a prognostic marker in thyroid carcinoma, controlling for the presence of *BRAF(V600E)* mutations.

Materials and Methods

Study Design

The study was undertaken with full approval of the Emory University Institutional Review Board (IRB) and the Collaborative IRB Training Initiative Program (Atlanta, GA). The Emory University Hospital Department of Pathology electronic database was queried for patients undergoing resection of thyroid carcinoma between 2002 and 2011. 81 patients with sufficient tissue for TMA IHC and molecular analysis were randomly chosen.

Final pathology reports and medical records were reviewed to determine patient age, gender, tumor size, histologic subtype, pathologic stage, tumor capsule invasion (TCI), ETE, margin status, lymph-vascular invasion (LVI), LNM, and distant metastases at presentation. Disease recurrence was defined as recurrence of the same histologic carcinoma necessitating additional therapy or surgery, or elevated thyroglobulin levels with documented radiographic evidence of disease after a disease-free interval. Persistent disease requiring a second radioactive iodine treatment was not considered a recurrence. Local recurrence was defined as tumor recurrence in the thyroid bed or neck lymph nodes and distant recurrence was at all other sites. Disease specific survival was determined from documented clinical follow-up in each patient's medical record and calculated from the date of surgery.

Tissue Microarray (TMA) and Immunohistochemistry (IHC)

Hematoxylin and eosin stained sections from 81 formalin-fixed paraffin embedded blocks of PTC (n=39), FTC (n=27), MTC (n=9), and ATC (n=6) were reviewed by a blinded staff pathologist (CC) to confirm the diagnosis. Three TMAs were constructed using two, 1.0 mm diameter core samples from morphologically representative tissue. Normal thyroid tissue

(two cores) was included as a control. IHC using standard heat-induced epitope retrieval in citrate buffer pH 6 was performed. Slides of the TMAs were loaded on the DAKO Autostainer (Dako, Carpinteria, CA), exposed to 3% hydrogen peroxide for 5 minutes, primary monoclonal antibody to EGFR (Clone 2-18C9, Dako) for 30 minutes, labeled with polymer horseradish peroxidase (Envision+dual link; Dako) for 30 minutes, diaminobenzidine as a chromogen for 5 minutes, and hematoxylin as a counterstain for 5 minutes. Incubations were performed at room temperature, and between incubations sections were washed with Tris-buffered saline. Coverslipping used the Tissue-Tek SCA coverslipper (Sakura Finetek USA, Inc. Torrance, CA). A known EGFR 3+ positive control (colon carcinoma) and negative controls (buffer, no primary antibody) were included in each run.

TMA IHC Scoring

One of two pathologists (JCJ or CC) scored the TMAs for membranous EGFR IHC intensity (0–3+) and percent positivity (0–100%), and all cases were scored again by a third pathologist (KEF) using light microscopy. The reported percent positivity is the average of the two percentage scores. All pathologists were blinded to clinicopathologic variables and outcomes while assessing IHC results. The scoring system was modeled after one previously used at our institution designed to account for both staining intensity and percentage of cells staining (25, 26). No EGFR expression was defined as 0+ staining intensity in all tumor cells. Low EGFR was defined as 1+ staining of any percentage of tumor cells or 2+ staining of <50% of tumor cells. EGFR overexpression (EGFR-H) was defined as 3+ staining of any percentage of tumor cells or 2+ staining of ≥50% of tumor cells. No inter-observer scoring discrepancies occurred that would have re-classified the tumor from one EGFR category to another (e.g. from absent to low EGFR expression or low EGFR expression to EGFR-H).

Molecular Analysis

A carcinoma-containing paraffin block from each case used in the TMA was selected for mutational analysis. DNA was extracted using a Qiagen DNA Mini Extraction Kit (Qiagen Inc. Valencia, CA) per the manufacturer's protocol. DNA from each individual case was amplified using the following conditions. Hot start: 95°C 15 minutes (all); Denature: 95°C for 20 seconds (all); Primer anneal: 65°C for 30 seconds (*EGFR* exon 19), 60°C for 30 seconds (*EGFR* exon 21), 55°C for 20 seconds (*BRAF*); Extension: 72°C for 20 seconds (*EGFR* exon 19 and *BRAF*), 72°C for 30 seconds (*EGFR* exon 21), Cycles: 42 (*EGFR* exon 19 and *BRAF*), 43 (*EGFR* exon 21); Final extension: 72°C for 5 minutes (all). The following primers (Integrated DNA Technologies, Coralville, IA) were used for strand amplification: *EGFR* exon 19 FW: 5'-AGAAAGTTAAAATTCCCGTCGCTAT-3', *EGFR* exon 19 REV: 5'-CCACAGCAAAGCAGAACTCACAT-3'; *EGFR* exon 21 FW: 5'-GAAAACACCGCAGCATGTCAA-3', *EGFR* exon 21 REV: 5'-CCTCCTTCTGCATGGTATTCTTTC-3'; *BRAF* FW: 5'-TGAAGACCTCACAGTAAAAATAGG-3', *BRAF* REV: 5'-TCCAGACAACTGTTCAAAGTAT-3'. The T>A single base pair mutation at amino acid 1799 (codon 600) in exon 15 of *BRAF* and the T>G single base pair mutation at amino acid 2573 (codon 858) in exon 21 of *EGFR* were analyzed using pyrosequencing with a Qiagen Pyromark Q96 (Qiagen Inc.). Peaks of interest were compared to wild-type sequence and

tumors with known positive mutations (melanoma for *BRAF(V600E)* and lung adenocarcinoma for *EGFR*). Deletions in exon 19 of *EGFR* spanning amino acids 2235-2250 (codons 746-750) were determined by running capillary electrophoresis and comparing the bands to known base pair size markers and compared to a known positive lung adenocarcinoma. Reference laboratory samples positive for the mutation of interest were used as positive controls. Mutation status interpretation was performed by one pathologist and confirmed by a second (CF and CEH or KEF).

Statistical Analysis

Statistical analysis was performed using SPSS version 20 (IBM, New York, NY). Fisher exact or Pearson chi-square tests were used to compare categorical variables; independent sample t-tests were used to compare continuous clinicopathologic parameters. When sample sizes were small and non-normally distributed the non-parametric Kruskal-Wallis test was used to compare continuous clinicopathologic parameters. Statistical significance was predefined at a p-value <0.05. Kaplan Meier survival analyses were used to assess recurrence-free and overall survival. Pathologic factors significant to a level of p<0.1 on Cox univariate regression were included in a multivariate model to assess association between EGFR-H and other adverse pathologic features. In the case of multicollinearity between covariates the single variable that best described the clinical entity was selected for the model.

Results

Clinical Characteristics of All Patients (n=81)

81 patients were identified, 34.6% (n=28) were male. The median age was 49.0 (range 14.2-84.0). The median follow-up was 35.3 months (range 0.0-128.0 months), during which 12 patients (14.8%) experienced local (n=7, 8.6%) or distant (n=5, 6.2%) disease recurrence. The mean recurrence-free survival was 98.2 months (median not reached). The mean disease specific survival was 112.0 months (median not reached). The pathologic characteristics for all patients undergoing thyroidectomies are summarized in Table 1. Data were missing for margin status (n=1), TCI (n=6), ETE (n=5), and LVI (n=3). When evaluating the prognostic role of LNM, patients who did not undergo lymph node sampling (n=35) were excluded from analysis.

EGFR and *BRAF* Analyses in All Patients

EGFR overexpression (EGFR-H) was detected in 39.5% of carcinomas (n=32) from patients with PTC, FTC, and ATC, but not MTC (Table 1). Representative images of EGFR expression (absent, low, and overexpression) in PTC, FTC, MTC, ATC, or control thyroid tissue are shown in Figure 1. No *EGFR* exon 19 deletions or *EGFR* exon 21 activating mutations were observed in any tumor. *BRAF(V600E)* mutations were identified in 22.2% of carcinomas (n=18): 15 PTCs, 3 ATCs, and no FTCs or MTCs (Table 1). On univariate analysis of all patients, EGFR-H did not correlate with any pathologic feature (increasing tumor size, TCI, ETE, positive margins, LVI, LNM, distant metastases, pathologic T stage, or AJCC 7th edition stage).

EGFR-H and *BRAF(V600E)* Mutations in Papillary Thyroid Carcinoma (n=39)

For PTC patients, the median age was 45.6 years (range 21.6-78.2) and 30.8% (n=12) were male. 46.2% of tumors (n=18) exhibited classical histology, 15.4% (n=6) were follicular variant of PTC (FV-PTC), one (2.6%) tumor was classified as Warthin-like variant, and in the remaining 35.9% of tumors (n=14) the histologic variant was not otherwise specified (NOS). 20.5% PTCs (n=8) did not express EGFR, 33.3% (n=13) demonstrated low EGFR expression, and 46.1% (n=18) had EGFR-H. As EGFR expression increased, tumor size and the proportion of PTCs with positive margins, advanced stage, LVI, LNM, and distant metastases all increased (Table 2). When stratified by EGFR IHC expression, 57.1% (n=16) of patients with adverse pathologic features (APF, defined as any demonstration of ETE, TCI, LVI, LNM, and/or distant metastases) had tumors with EGFR-H (Table 2).

On univariate analysis of PTC patients, EGFR-H correlated with increasing pathologic T (pT) stage, TCI, ETE, *BRAF(V600E)* mutation, and APF (Table 3). EGFR-H expression was not associated with sex, age, tumor size, AJCC 7th edition stage, histologic variant, margin status, LVI, LNM, or distant metastases ($p>0.05$). On multivariate analysis, EGFR-H correlated with *BRAF(V600E)* mutations, although the analysis was limited by the small sample size (Table 3). Ten patients (25.6%) had tumors with both EGFR-H and *BRAF(V600E)* mutations.

BRAF(V600E) mutations were present in 61.1% (11/18) of classical variant PTCs and 28.6% (4/14) of PTC-NOS. No mutations were detected in the 6 FV-PTCs or the one Warthin-like variant. On univariate analysis, *BRAF(V600E)* mutations were associated with EGFR-H and the classical histologic variant ($p=0.010$) but not with other pathologic factors, including APF.

Recurrence and Disease-Specific Survival in Patients with Papillary Thyroid Carcinoma

Given the small sample size, low rate of recurrence (n=7), and low disease-specific mortality (n=2), formal survival analyses were limited. Of the seven patients with PTC who experienced recurrence, 6 (83.3%) had tumors that expressed some level of EGFR by IHC, and 4 (57.1%) had EGFR-H. *BRAF(V600E)* mutations were present in two patients, and one patient had both EGFR-H and *BRAF(V600E)* mutation. All seven patients who experienced recurrence had APF, and the correlation between APF and recurrence approached statistical significance ($p=0.051$). One patient with metastatic disease at presentation died of pulmonary complications related to tumor burden 6 months after thyroidectomy despite surgical intervention and radioactive iodine therapy. The other PTC patient who died also initially presented with metastatic disease and recurred after a disease-free interval. Both patients had tumors with EGFR-H and *BRAF(V600E)* mutation.

EGFR is Overexpressed in *BRAF* Wild-type Papillary Thyroid Carcinomas with Adverse Pathologic Features

APF were present in 28 patients (71.8%) with PTC. Of these 28, 16 (57.1%) demonstrated EGFR-H by IHC, 11 (39.3%) harbored *BRAF(V600E)* mutations, 18 (64.3%) had either EGFR-H by IHC or a *BRAF(V600E)* mutation, 9 (32.1%) had both, and 10 (35.7%) had neither. Of the 17 patients with *BRAF*-WT PTCs with APF, 7 had EGFR-H (41.2%). There

were no discernible clinicopathologic differences between the 10 patients with *BRAF*-WT, non-EGFR-H tumors with APF and the 7 patients with *BRAF*-WT, EGFR-H tumors with APF. The clinicopathologic details of the 39 patients grouped by EGFR expression are provided in Supplemental Table 1.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for EGFR-H, *BRAF*(V600E), or combinations thereof to predict APF are shown in Table 4. The sensitivity, specificity, PPV, and NPV of EGFR-H for APF were all higher than *BRAF*(V600E) alone (57.1% vs. 39.3%, 81.8% vs. 63.3%, 88.9% vs. 73.3%, and 42.9% vs. 29.2%, respectively), and the combination of EGFR-H and *BRAF*(V600E)-positivity yielded slightly better sensitivity for APF compared to EGFR alone (64.3% vs. 57.1%). In *BRAF*-WT PTCs, EGFR-H had 100.0% specificity and PPV for APF. The correlation between EGFR-H and APF in *BRAF* WT PTCs approached statistical significance ($p=0.065$).

Discussion

In this study, we demonstrate that patients with PTCs, FTCs, and ATCs, but not MTCs, express EGFR by IHC despite the absence of two common somatic mutations (exon 19 deletions or exon 21 point mutations). Others have also shown absence of identifiable *EGFR* mutations in PTCs, FTCs, MTCs, and ATCs (10, 12, 15), although Masago *et al.* identified these mutations in 30% ($n=7$) of Japanese patients with PTC (13). It is possible that the different ethnicities in the patient populations tested may be responsible for our discrepant results. East Asian patients with non small cell lung carcinoma are more likely to harbor *EGFR* mutations than other ethnicities and this finding may extrapolate to thyroid carcinomas (27). Additionally, most *EGFR* mutation assays are based on the frequent mutations observed in lung adenocarcinoma, though over 40 activating mutations have been described (11). It is possible that thyroid carcinomas harbor an *EGFR* kinase domain mutation that is seen infrequently or is not present in lung adenocarcinomas, or, as our data and that of others imply (10, 12, 15), that *EGFR* somatic mutations are not required for membranous EGFR-H by IHC. Similar discordance between EGFR IHC expression and the molecular mutation status has been described in lung adenocarcinomas (28).

Although others have reported overexpression of EGFR in PTC (29) as well as FTC (30) and ATC (5, 31, 32), to our knowledge, this is the first clinical study to demonstrate a relationship between EGFR expression and multiple adverse pathologic features in PTC. In a recent study, Landriscina *et al.* demonstrated that EGFR-H is associated with areas of dedifferentiation in tissue samples from patients with poorly differentiated thyroid carcinoma or ATC (5). In the current study no patient with PTC demonstrated dedifferentiation but all patients with ATC had EGFR-H. Regarding PTC, tumors with EGFR-H tend to be locally aggressive as evidenced by the statistically significant association with higher pathologic stage, TCI, and ETE. Additionally, EGFR-H identifies PTC patients with APF, and EGFR-H may be a particularly useful prognostic marker for APF in patients with *BRAF*-WT PTC. The association between EGFR expression and disease severity in our study supports others' hypotheses that EGFR overexpression is a hallmark of an aggressive phenotype (5, 33).

In the current study the percentage of patients with PTCs harboring *BRAF(V600E)* mutations is consistent with previous reports (18, 21). In our data set, *BRAF(V600E)* mutations did not correlate with APF, suggesting that while *BRAF(V600E)* status confers some prognostic information, it is not a comprehensive marker. Our data suggest that in patients with *BRAF*-WT PTC, EGFR-H offers prognostic utility for APF with high specificity and PPV. Since limited literature exists regarding prognostic markers in patients with *BRAF*-WT PTCs, the finding that EGFR-H status predicts the presence of APF could help guide surgical and/or medical management, although further testing and validation is required in larger, prospective studies. The prognostic role for EGFR-H and disease recurrence also requires further investigation.

Classically, EGFR overexpression and *BRAF* mutations are thought to be mutually exclusive: tumors that overexpress EGFR tend to express wild-type *BRAF*, *RAS*, and other MAPK effectors given that these oncogenes share similar signaling pathways (34, 35). However, the current work documents an unexpected finding of concomitant EGFR-H and *BRAF(V600E)* mutations in a quarter of patients with PTC, and the presence of a *BRAF(V600E)* mutation is associated with EGFR-H by IHC on multivariate analysis. This “dual-oncogene” phenotype, recently elucidated by Prahallad and colleagues in *in vitro* and murine xenograft models (36), has important therapeutic implications; tumors harboring both EGFR-H and *BRAF* mutations were sensitive only to combination EGFR/*BRAF* inhibitor therapy, but not monotherapy in their models. It also provides some insight into the failure of a recent phase II trial testing gefitinib monotherapy (an EGFR inhibitor) for patients with advanced thyroid carcinoma that showed heterogeneous response rates and no statistically significant trend in overall response rate (37). The trial included patients with tumors of multiple histologic subtypes, and neither the immunohistochemical expression pattern of EGFR nor the mutation status of *EGFR* or *BRAF* in each tumor was known. It is possible that stratifying the responses based on EGFR expression and *BRAF(V600E)* mutation status may reveal an effect. To better control for the interplay between EGFR expression and *EGFR* and/or *BRAF* mutation status, future clinical trials testing anti-EGFR therapy in thyroid malignancies should incorporate the morphologic, IHC, and molecular status of the tumor to accurately assess therapeutic benefit. Routine tissue-based testing prior to targeted therapy administration should be strongly considered (38); when not feasible, the authors encourage tissue-banking for retrospective analysis of tumor markers. Given the heterogeneity (or absence) of *EGFR* mutations, perhaps alternate methods of identifying mutations such as whole exome or whole genome sequencing should be investigated.

The subjective nature of IHC is a limitation which is not unique to the current study, given that no validated IHC scoring system for EGFR expression exists. Previous works have considered positive staining for EGFR any percentage of tumor cells expressing circumferential cellular staining of moderate or strong intensity (5), while in ATCs others have defined positive as 10% of tumor cells with membranous staining of any intensity (6). Our IHC scoring algorithm is modeled after other studies from our institution (25, 26) and accounts for both the intensity and percentage of cells staining to buffer for tumor heterogeneity and variations in staining technique. Future research is needed to determine the ideal method for assessing EGFR expression in thyroid carcinomas.

We also acknowledge that our study is limited by its retrospective nature and the relatively small number of cases available for statistical analysis. The small sample size may explain why *BRAF(V600E)* mutations did not correlate with APF, and why there were no discernible differences in APF between patients with *BRAF*-WT PTCs that did not overexpress EGFR, and patients with *BRAF*-WT PTCs that overexpressed EGFR. As noted previously, the small sample size also precludes thorough analysis of recurrence or survival in our data set.

In summary, EGFR overexpression is a marker for locally aggressive PTCs and may have prognostic utility in *BRAF*-WT PTCs. Somatic *EGFR* mutations in exon 19 or exon 21 are not detected in PTCs, FTCs, MTCs, or ATCs suggesting that EGFR-H is not due to common mutations of the *EGFR* kinase domain. Although EGFR-H and *BRAF(V600E)* mutations are traditionally thought to be mutually exclusive (34, 35), we demonstrate that EGFR-H and *BRAF(V600E)* mutations coexist in human PTC and ATC, which has important therapeutic implications. We conclude that EGFR IHC testing identifies a subset of patients with aggressive thyroid carcinomas not detected by *BRAF(V600E)* or *EGFR* mutational analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Representative Images of EGFR Staining by Immunohistochemistry in Thyroid Carcinoma

Five micron sections of 1 mm TMA cores stained with a primary monoclonal antibody to EGFR (Clone 2-18C9). EGFR overexpression (EGFR-H) was defined as 3+ staining of any percentage of tumor cells or 2+ staining of 50% of tumor cells. PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, MTC: medullary thyroid carcinoma, ATC: anaplastic thyroid carcinoma, CTRL: control normal thyroid tissue. Magnification: 400×

Table 1

Pathologic Characteristics, All Patients

Characteristic	Total (n = 81) n (%)	PTC (n = 39) n (%)	FTC (n = 27) n (%)	MTC (n = 9) n (%)	ATC (n = 6) n (%)
Median tumor size (cm)	2.5	1.7	5.0	1.0	4.2
Tumor capsular invasion (TCI)	28 (37.3%)	15 (45.5%)	12 (44.4%)	0 (0.0%)	1 (16.7%)
Extrathyroidal extension (ETE)	20 (26.3%)	11 (30.6%)	3 (12.0%)	1 (11.1%)	5 (83.3%)
Positive margins	14 (17.5%)	7 (18.4%)	3 (11.1%)	1 (11.1%)	3 (50.0%)
pT stage					
T1a/b	28 (34.6%)	19 (48.7%)	4 (14.8%)	5 (55.6%)	0 (0.0%)
T2	10 (12.3%)	4 (10.3%)	4 (14.8%)	2 (22.2%)	0 (0.0%)
T3	30 (37.0%)	13 (33.3%)	16 (59.3%)	1 (11.1%)	0 (0.0%)
T4a/b	13 (16.0%)	3 (7.7%)	3 (11.1%)	1 (11.1%)	6 (100.0%)
Lymph-vascular invasion (LVI)	40 (51.3%)	14 (38.9%)	20 (74.1%)	2 (22.2%)	4 (66.7%)
Lymph node metastases (LNM)*	30 (65.2%)	21 (67.7%)	1 (20.0%)	5 (71.4%)	3 (100.0%)
Distant metastases	5 (6.2%)	2 (5.1%)	1 (3.7%)	0 (0.0%)	2 (33.3%)
AJCC 7 th edition stage					
I	45 (55.6%)	28 (71.8%)	13 (48.1%)	4 (44.4%)	0 (0.0%)
II	11 (13.4%)	1 (2.6%)	9 (33.3%)	1 (11.1%)	0 (0.0%)
III	9 (11.1%)	6 (15.4%)	2 (7.4%)	1 (11.1%)	0 (0.0%)
IV A-C	16 (19.8%)	4 (10.2%)	3 (11.1%)	3 (33.3%)	6 (100.0%)
EGFR-H	32 (39.5%)	18 (46.2%)	8 (29.6%)	0 (0.0%)	6 (100.0%)
<i>BRAF</i> (V600E) mutation	18 (22.2%)	15 (38.5%)	0 (0.0%)	0 (0.0%)	3 (50.0%)

* Percentages reported exclude pNx patients (n=35)

PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, MTC: medullary thyroid carcinoma, ATC: anaplastic thyroid carcinoma, EGFR-H: 3+ staining of any percentage of tumor cells or 2+ staining of 50% of tumor cells.

Table 2
EGFR Expression in Papillary Thyroid Carcinoma Patients

Characteristic PTC (n=39)	No EGFR (n=8) n (%) Total	Low EGFR (n=13) n (%) Total	EGFR-H (n=18) n (%) Total	p-value *
Median tumor size (cm)	1.8 cm	2.0 cm	2.3 cm	0.821 **
Tumor capsule invasion (TCI)	2 (6.1%)	3 (9.1%)	10 (30.3%)	0.040
Extrathyroidal extension (ETE)	0 (0.0%)	2 (5.6%)	9 (25.0%)	0.012
Positive margins	1 (2.6%)	2 (5.3%)	4 (10.5%)	1.000
pT stage				
T1a/b	5 (12.8%)	8 (20.5%)	6 (15.4%)	0.373
T2	1 (2.6%)	2 (5.1%)	1 (2.6%)	
T3	2 (5.1%)	2 (5.1%)	9 (23.1%)	
T4a/b	0 (0.0%)	1 (2.6%)	2 (5.1%)	
Lymph-vascular invasion (LVI)	1 (2.8%)	4 (11.1%)	9 (25.0%)	0.237
Lymph node metastases (LNM) [§]	2 (6.5%)	6 (19.4%)	13 (41.9%)	0.206
Distant metastases	0 (0.0%)	0 (0.0%)	2 (5.1%)	0.684
AJCC 7 th edition stage				
I	8 (20.5%)	10 (25.6%)	10 (25.6%)	0.461
II	0 (0.0%)	0 (0.0%)	1 (2.6%)	
III	0 (0.0%)	2 (5.1%)	4 (10.3%)	
IVA-C	0 (0.0%)	1 (2.6%)	3 (7.7%)	
Adverse pathologic features (APF)	3 (7.7%)	8 (20.5%)	16 (41.0%)	0.019

* Fisher's exact tests were used to compare the groups except as designated by

**, in which a Kruskal-Wallis test was used

[§] Percentages reported exclude pNx patients (n=8)

No EGFR: 0+ staining intensity in all tumor cells. Low EGFR: 1+ intensity in any percentage of tumor cells or 2+ intensity in <50% of tumor cells. EGFR-H: 3+ staining of any percentage of tumor cells or 2+ staining of 50% of tumor cells.

Table 3
Univariate and Multivariate Analysis of EGFR-H in Papillary Thyroid Carcinoma

Pathologic Factor	Univariate	Multivariate		
	p-value	HR	95% CI	p-value
LVI*	0.107	-	-	-
LNM*	0.106	-	-	-
Distant metastases*	0.622	-	-	-
TCI*	0.014	-	-	-
ETE*	0.007	-	-	-
Increasing pT stage	0.040	2.08	0.86-5.03	0.103
<i>BRAF(V600E)</i> mutation	0.010	13.45	1.93-93.8	0.009
APF	0.022	6.12	0.74-50.7	0.093

* Excluded from multivariate model due to multicollinearity with APF

LVI: lymph-vascular invasion, LNM: lymph node metastases, TCI: tumor capsule invasion, ETE: extrathyroidal extension, pT stage: pathologic T stage, APF: adverse pathologic features

Table 4
Ability of EGFR-H or *BRAF*(V600E) Mutation to Identify Papillary Thyroid Carcinoma Patients with Adverse Pathologic Features

EGFR-H identifies *BRAF*-WT PTC patients with APF

Characteristic (all patients with PTC, n=39)	Sensitivity	Specificity	PPV	NPV
EGFR-H	57.1%	81.8%	88.9%	42.9%
<i>BRAF</i>(V600E)	39.3%	63.3%	73.3%	29.2%
EGFR-H and/or <i>BRAF</i>(V600E)	64.3%	63.6%	81.8%	41.2%
EGFR-H in <i>BRAF</i>(V600E) patients (n=15)	81.8%	50.0%	81.8%	50.0%
EGFR-H in <i>BRAF</i>-WT patients (n=24)	41.2%	100.0%	100.0%	41.2%

PPV: positive predictive value, NPV: negative predictive value, *BRAF*-WT: *BRAF* wild-type