Lung Cancer 81 (2013) 138–141

Contents lists available at SciVerse ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Early report

Nuclear EGFR protein expression predicts poor survival in early stage non-small cell lung cancer

Anne M. Traynor[a], Tracey L. Weigel[b], Kurt R. Oettel[c], David T. Yang[d], Chong Zhang[e], KyungMann Kim[e], Ravi Salgia[f], Mari Iida[g], Toni M. Brand[g], Tien Hoang[a], Toby C. Campbell[a], Hilary R. Hernan[a], Deric L. Wheeler[g,*]

[a] Department of Medicine and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[b] Department of Surgery and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[c] Gundersen Lutheran Center for Cancer & Blood Disorders, Gundersen Lutheran Medical Center, LaCrosse, WI, USA
[d] Department of Pathology and Laboratory Medicine and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[e] Department of Biostatistics and Medical Informatics and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[f] Department of Medicine and Cancer Research Center, Pritzker School of Medicine, University of Chicago, Chicago, IL, USA
[g] Department of Human Oncology and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 15 February 2013
Accepted 28 March 2013

Keywords:
Non-small cell lung cancer
Nuclear
Epidermal growth factor receptor
Prognosis
Biomarker
Survival analysis

ABSTRACT

Introduction: Nuclear EGFR (nEGFR) has been identified in various human tumor tissues, including cancers of the breast, ovary, oropharynx, and esophagus, and has predicted poor patient outcomes. We sought to determine if protein expression of nEGFR is prognostic in early stage non-small cell lung cancer (NSCLC).
Methods: Resected stages I and II NSCLC specimens were evaluated for nEGFR protein expression using immunohistochemistry (IHC). Cases with at least one replicate core containing ≥5% of tumor cells demonstrating strong dot-like nucleolar EGFR expression were scored as nEGFR positive.
Results: Twenty-three (26.1% of the population) of 88 resected specimens stained positively for nEGFR. Nuclear EGFR protein expression was associated with higher disease stage (45.5% of stage II vs. 14.5% of stage I; $p = 0.023$), histology (41.7% in squamous cell carcinoma vs. 17.1% in adenocarcinoma; $p = 0.028$), shorter progression-free survival (PFS) (median PFS 8.7 months [95% CI 5.1–10.7 mo] for nEGFR positive vs. 14.5 months [95% CI 9.5–17.4 mo] for nEGFR negative; hazard ratio (HR) of 1.89 [95% CI 1.15–3.10]; $p = 0.011$), and shorter overall survival (OS) (median OS 14.1 months [95% CI 10.3–22.7 mo] for nEGFR positive vs. 23.4 months [95% CI 20.1–29.4 mo] for nEGFR negative; HR of 1.83 [95% CI 1.12–2.99]; $p = 0.014$).
Conclusions: Expression of nEGFR protein was associated with higher stage and squamous cell histology, and predicted shorter PFS and OS, in this patient cohort. Nuclear EGFR serves as a useful independent prognostic variable and as a potential therapeutic target in NSCLC.

1. Introduction

Non-small cell lung cancer is a heterogeneous malignancy, comprised of multiple histologic subtypes. Predicting the course of disease based upon staging is suboptimal. The identification of biological markers of aggressive clinical behavior is needed in an effort to individualize treatment and develop novel therapeutic targets.

Protein expression of membrane bound EGFR was neither prognostic nor predictive of efficacy with the use of erlotinib, gefitinib, or cetuximab in NSCLC [1,2]. However, emerging preclinical and clinical evidence supports the role of nEGFR in enhancing tumor cell growth, survival, and resistance to systemic and radiation therapies [3–10]. Herein, we report identification of nEGFR protein expression as an independent prognostic variable in early stage NSCLC.

2. Materials and methods

2.1. Patients and specimen collection

For this retrospective analysis of patients who underwent curative intent resections, de-identified tumor specimens from 88 deceased patients with stages I and II NSCLC were collected from the University of Wisconsin Hospitals and Clinics (UWHC; Madison, WI) and from the Gundersen Lutheran Medical Center (GLMC;

* Corresponding author at: Department of Human Oncology and Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, 3159 WIMR, 1111 Highland Avenue, Madison, WI 53705, USA. Tel.: +1 608 262 7837; fax: +1 608 263-9947.
E-mail address: dlwheeler@wisc.edu (D.L. Wheeler).

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http://dx.doi.org/10.1016/j.lungcan.2013.03.020

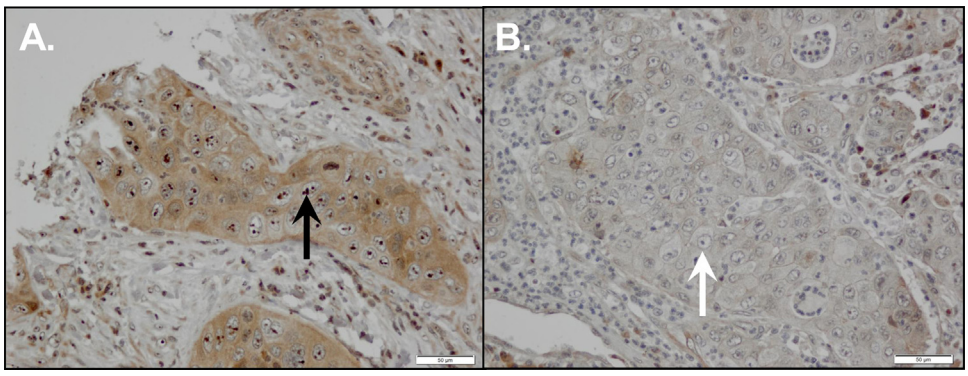

Fig. 1. Nuclear EGFR (nEGFR) is detected in early stage NSCLC specimens. We analyzed 88 primary NSCLC tumors for nEGFR protein expression using immunohistochemistry. (A) Representative case demonstrating nEGFR expression. All positive cases had a similar distinctive pattern of strong nucleolar staining (black arrow). (B) Representative case demonstrating a lack of nEGFR protein expression. Despite the presence of prominent nucleoli, no nEGFR protein is detected (white arrow).

LaCrosse, WI). Patients did not receive either pre- or post-operative anti-cancer therapy. We also collected: age, sex, histology, smoking history, pathologic stage (AJCC Staging 6th edition), type of resection, date of relapse, and date of death. Approval for this research was obtained from the IRBs of UW-Madison and the GLMC.

2.2. Tissue microarray construction and protein expression analyses

Tumor tissue quality and pathology were confirmed by the study pathologist (DTY). Tissues were harvested within 30 min of resection, fixed with 10% neutral buffered formalin and embedded in paraffin. Areas of tumor and adjacent benign tissue were marked on a representative H & E stained section. Duplicate 0.6 mm cores from the corresponding paraffin block were punched out and assembled with a Manual Tissue Arrayer (Beecher Instruments, Sun Prairie, WI).

For nEGFR protein expression analyses, tissue sections were deparaffinized and antigen retrieval was performed in citrate buffer (pH 6.0) with 0.05% Tween-20. Samples were incubated with EGFR polyclonal antibody (sc-03, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4C. Samples were washed and incubated in secondary antibody for 1 hour followed by incubation with Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA, USA). 3,3-Diaminobenzidine staining was used as the color-developing reagent. Slides were counterstained with Mayer hematoxylin, dehydrated through a graded series of ethanol washes to xylene, and coverslipped with Permount (Fisher, Springfield, NJ).

We initially hypothesized that assessment of nEGFR protein would require the quantitative and subcellular localization capacity of automated quantitative analysis (AQUA). When we observed that the nuclear staining of EGFR protein revealed a distinct, robust nucleolar pattern (Fig. 1A) that clearly contrasted with negative cases (Fig. 1B) using routine IHC staining, we switched to the IHC methodology due to its easier translation to clinical practice. The nEGFR staining pattern was scored by the study pathologist at 5% increments by visual estimation at 20× magnification. Accordingly, cases with at least one replicate core containing at least 5% of tumor cells demonstrating strong dot-like nucleolar EGFR IHC protein expression were scored as nEGFR positive.

2.3. Statistical analyses

Our endpoints were protein expression of nEGFR and PFS and OS. Originally this study had an approximate power of 0.902, 0.747 and 0.477 to detect a hazard ratio of 2, 1.75 and 1.5, respectively, using a two-sided log-rank test at a significance level 0.05, given the sample size of 88 when the AQUA score was dichotomized using its median. The prognostic impact of nEGFR was assessed using the log-rank test and Cox proportional hazards regression models for PFS and OS. Kaplan–Meier method was used to summarize PFS and OS for patients per nEGFR IHC. Association between nEGFR protein expression and sex, histology, smoking history and pathologic stage was assessed using Fisher's exact test.

3. Results

3.1. Patient characteristics

Table 1 summarizes the characteristics of the 88 patient samples studied. None of the patients received either pre- or post-operative anti-cancer therapy. The median PFS and OS for our population

Table 1
Patient characteristics.

	N
Number of patients	88
Median age (range)	73 (43–96 yrs)
Sex	
Male	55 (62.5%)
Female	33 (37.5%)
Histology	
Adenocarcinoma	41 (46.6%)
Squamous cell	36 (40.9%)
Bronchioloalveolar	4 (4.5%)
Large cell	3 (3.4%)
Non-small cell, NOS	2 (2.3%)
Adenosquamous carcinoma	2 (2.3%)
Smoking history	
Current or former	84 (95.5%)
Type of surgery	
Lobectomy	80 (90.9%)
Pneumonectomy	7 (8%)
Bilobectomy	1 (1.1%)
Disease stage	
IA	23 (26.1%)
IB	32 (36.4)
IIA	9 (10.2%)
IIB	24 (27.3%)
T stage	
T1	31 (35.2%)
T2	52 (59.1%)
T3	5 (5.7%)
N stage	
N0	60 (68.2%)
N1	28 (31.8%)
Nuclear EGFR protein expression	
Positive	23 (26.1%)