Evaluating the epidermal growth factor receptor in non small cell carcinoma of lung according to the grade of tumor and cellular differentiation

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Abstract: Small cell lung cancer (NSCLC) which comprises the majority of lung cancer has proven difficult to treat due to poorly understood pathological mechanisms. Over expression of EGFR has been reported and implicated in the pathogenesis of many human malignancies, including NSCLC. The aim of this study was to evaluate the epidermal growth factor receptor in non small cell carcinoma of lung according to the grade of tumor and cellular differentiation. In this retrospective study, 50 patients with primary lung carcinoma diagnosed as pathologic NSCLC who underwent complete surgical resection with systematic lymph node dissection without adjuvant chemotherapy were evaluated. Patient gender, age, tumor size, tumor location, surgical procedure, pathologic TNM stage, and patient outcome were determined from the medical records. There were 40 males and 10 females with a mean age of 55.12 ± 10.14 years. Most patients (40 patients; 80%) had adenocarcinoma (ADC), whereas 8 patients (16%) had squamous cell carcinoma (SCC), 2 patients (4%) had LCNECs. EGFR expression was not associated with age, sex, smoking status, pathologic stage, or tumor or node status. Significant differences were associated with histologic differentiation, with well-differentiated tumors expressing higher levels of EGFR than the poorly differentiated tumors. A statistically significant difference in the EGFR expression was observed across the histological subtypes (P< .001). We conclude that most of the NSCLC tumors have high EGFR expression, especially in the SCC subtypes.

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1. Introduction

Non-small cell lung cancer (NSCLCs) is the most common type of lung cancer. About 85% of lung cancers are non-small cell lung cancers. As a class, NSCLCs are relatively insensitive to chemotherapy, compared to small cell carcinoma. (Knetki-Wroblewska et al. 2012; Mekic-Abazovic et al. 2012) When possible, they are primarily treated by surgical resection with curative intent, although chemotherapy is increasingly being used both pre-operatively and post-operatively.(Lotti et al. 2013; Goldberg et al. 2012) The most common types of NSCLC are squamous cell carcinoma, large cell carcinoma, and adenocarcinoma, but there are several other types that occur less frequently, and all types can occur in unusual histologic variants and as mixed cell-type combinations.(Vazquez-Martin et al. 2013; Mimeault and Batra 2013) Sometimes the phrase "non-small-cell lung cancer" is used generically, usually when a more specific diagnosis cannot be made. This is most often the case when a pathologist examines a small amount of malignant cells or tissue in a cytology or biopsy specimen. Lung cancer in never-smokers is almost universally NSCLC, with a sizeable majority being adenocarcinoma. (Mohebbipour et al. 2012; Minami et al. 2012) On relatively rare occasions, malignant lung tumors are found to contain components of both SCLC and NSCLC. In these cases, the tumors should be classified as combined small cell lung carcinoma and are treated like "pure" SCLC. Progress in lung cancer biology led to the development of small molecule inhibitors of target proteins involved in proliferation, apoptosis, and angiogenesis.(Campbell et al. 2013; Minuti et al. 2013) The epidermal growth factor receptor (EGFR) superfamily, including the four distinct receptors EGFR/erbB-1, HER2/erbB-2, HER3/erbB-3, and HER4/erbB-4, was identified early as a potential therapeutic target in solid tumors. These receptors play an important role for tumor cell survival and proliferation.(Cadranel et al. 2013; Umekawa et al. 2013) EGFR overexpression has also been demonstrated in premalignant bronchial epithelium, suggesting that EGFR plays an important role in lung carcinogenesis. In lung carcinomas, EGFR is more commonly overexpressed than HER2/neu. (Wang et al. 2013; Hsiao et al. 2013) The prognostic association of EGFR overexpression in lung cancer, however, is a controversial issue. Several reports indicated that EGFR was associated with a poor prognosis, whereas no prognostic association was reported by other reports.(Liu et al. 2013; Abi-Jaoudeh et al. 2013) The EGFR levels were evaluated by immunohistochemistry (IHC) in

most studies, but other methods also have been reported. Different conclusions regarding prognostic significance may reflect differences in detection methods, reagents, assay cutoff points, and population characteristics.(Goldust et al. 2012; Li et al. 2012) This study aimed at evaluating the epidermal growth factor receptor in non small cell carcinoma of lung according to the grade of tumor and cellular differentiation.

2. Material and Methods

In this descriptive-analytical study conducted at We retrospectively analyzed clinical data from 50 patients with primary lung carcinoma diagnosed as pathologic NSCLC who underwent complete surgical resection with systematic lymph node dissection without adjuvant chemotherapy at Sina hospital, Tabriz from December 2011 to December 2012. This study was approve by ethic committee of Tabriz university of medical sciences. Written consent was obtained from all the patients. We excluded patients with limited surgical resection or incomplete resection. The pathologic NSCLC consisted of 40 adenocarcinomas, 8 squamous cell carcinomas, and 2 LCNECs. Patient gender, age, tumor size, tumor location, surgical procedure, pathologic TNM stage, and patient outcome were determined from the medical records. The sections for immunohistochemical analysis were deparaffinized through graded alcohols and xylene. Endogenous peroxidase was quenched with 3% hydrogen peroxide in methanol, and nonspecific binding was blocked with normal goat serum. An antigen retrieval method was used. In brief, the tissue sections were immersed in diluted, concentrated antigen-retrieval solution (BioGenex, San Ramon, CA, diluted 1:10) and heated to 100°C in a microwave oven (BioRad H2500 Microwave Processor) at 700 W (100%) for 10 minutes divided into two 5-minute cycles. After heating, the slides were left in the solution to cool for 15 minutes and then were rinsed in phosphate-buffered saline (PBS) (pH 7.4) for another 5 minutes. The sections were incubated overnight at 4°C with EGFR (1005) SC-03 polyclonal antibody (diluted 1:100; Santa Cruz Biotechonology, Inc, Santa Cruz, CA), c-neu (Ab-3) OP15 monoclonal antibody (1:100; Oncogen Research Products, Cambridge, MA), c-erbB-3 (RTJ.2) SC-415 monoclonal antibody (1: 100), and c-erbB-4 (C-18) SC-283 polyclonal antibody (1:100; Santa Cruz Biotechonology, Inc). The immunoreactivity was detected by the streptavidin-biotinperoxidase complex method (MultiLink SuperSensitive 500 Detection System, BioGenex). The chromogen was 3-33-diamino-benzidine (0.025%). The sections were counterstained with hematoxylin. Tumor cells with membranous and cytoplasmic staining were considered positive, and cells without any immunostaining were considered negative. The slides were reviewed independently by two pathologists who had no knowledge of the clinical outcome. The staining intensity of EGFR family members was ranked into four grades as follow: Cytoplasmic or without staining, Faint membrane, The whole membrane but <30 and the whole membrane but >30%.

Statistical Analysis

The Fisher exact test was used to compare binomial proportions. The chi square test was used to assess differences in gender, tumor site, and surgical methods. The unpaired *t* test was used to detect significant differences between patients with LCNEC and patients with other NSCLCs with respect to patient age, smoking index, and tumor size. $P<0.05$ was regarded as statistically significant.

3. Results

We studied 50 NSCLC patients with an average of 2.8 assessable tissue cores per patient. There were 40 males and 10 females with a mean age of 55.12 ± 10.14 years (range, 32 to 88 years). Smoking status was available for 40 patients. Most patients (40 patients; 80%) had adenocarcinoma (ADC), whereas 8 patients (16%) had squamous cell carcinoma (SCC), 2 patients (4%) had LCNECs. The majority of patients had poorly differentiated histology (54%), pathologic stage I (56%), and negative surgical margins (94%). The mean follow-up was 32 months from surgery. At the last follow-up, 15 patients had died of lung cancer and 35 patients were alive. EGFR expression was not associated with age, sex, smoking status, pathologic stage, or tumor or node status. Significant differences were associated with histologic differentiation, with well-differentiated tumors expressing higher levels of EGFR than the poorly differentiated tumors. A statistically significant difference in the EGFR expression was observed across the histological subtypes ($P < .001$). The EGFR expression was highest in SCC and lower in ADC. The majority (32 patients; 64%) had either intermediate (score 201 to 300; 14 patients; 26%) or high levels (score 301 to 400; 18 patients; 36%) of EGFR expression. Conversely, ADC and LCNECs had mostly negative or low levels of expression (58% and 68%, respectively). EGFR scoring was as follow: Cytoplasmic or without staining: 36 (72%), Faint membrane: 4 (8%), The whole membrane but <30%: 6 (12%) and the whole membrane but >30%: 4 (8%).

4. Discussions

Epidermal growth factor receptor is a trans-membrane glycoprotein with an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain that regulates signaling pathways to control cellular proliferation. Epidermal growth factor receptor binding to its ligand results in autophosphorylation by intrinsic tyrosine/kinase activity, triggering several signal transduction cascades. Constitutive or sustained activation of these sequences of downstream targets is thought to yield more aggressive tumor phenotypes.(Kelly et al. 2012; Ahn et al. 2012) Mutations in epidermal growth factor receptor have been discovered in association with some lung cancers. Lung adenocarcinomas with mutated epidermal growth factor receptor have significant responses to tyrosine kinase inhibitors, although for unselected patients it does not appear to have a survival benefit.(Hamada et al. 2012; Kim et al. 2012) However, in a subset of patients (non-smoking Asian women with adenocarcinoma, particularly with a bronchioloalveolar carcinoma), there appears to be a significant survival advantage. In the United States, about 15% of patients with non-small cell lung cancer have mutations to the EGFR.(Rosen et al. 2012; Rosell et al. 2012) Some research studies have shown that mutations to the EGFR may predict whether certain types of drugs, called tyrosine kinase inhibitors (TKIs), can help treat lung cancer. (Ciuleanu et al. 2012; Wada et al. 2012) Drobniene et al. demonstrated that EGFR testing helps to move toward the goal of tailoring treatments for the patient. They demonstrated that non-small cell lung cancer is many different diseases.(Drobniene et al. 2011) There is probably no more contentious or hotly debated topic related to lung cancer therapy than the question of how EGFR markers should be incorporated into clinical decision making. EGFR protein expression, EGFR gene copy number, and mutation status have been explored most extensively, and each may contribute information regarding which patients are likely to benefit from treatment.(Kaira et al. 2012; Ogasawara et al. 2011) Furthermore, EGFR markers may have different prognostic and predictive effects. Prognostic factors identify a better patient outcome that is independent of treatment and are best determined in untreated patients, whereas predictive factors identify a better outcome from treatment. (Murakami et al. 2012; Lee et al. 2012) This study demonstrated that the majority of NSCLC tumors exhibited either intermediate or high levels of EGFR protein expression, and there was a significant correlation between EGFR expression and histologic subtypes, with the highest expression in SCC and the lowest expression in ADC. Furthermore, the study showed that EGFR protein expression was more prominent in well-differentiated than in the poorly differentiated histology. The correlation between increasing levels of EGFR protein expression and increased gene copy number suggests that the additive effect of gene copies is an important mechanism for EGFR protein expression. We found a similar result regarding the HER2/neu expression in NSCLC patients. Preports utilizing Western blots or IHC have indicated that mutant protein may be present in approximately 15% of NSCLC tumors.(Sangha et al. 2011; Zhou et al. 2011) However, actual splice variant mRNA is not reported in lung cancer cell lines and the relevance of mutant or alternatively spliced *EGFR* mRNA remains to be determined. We also sought to determine whether *EGFR* gene or EGFR protein expression affected survival. The literature contains conflicting data on the relationship between EGFR expression and survival in lung cancer. This variability may be due to heterogeneity of study populations or lack of a standardized assay for determining EGFR status. (Kaira et al. 2011; Asami et al. 2011) After adjusting for prognostic factors as age, sex, stage, and surgical margins, patients with high gene copy numbers had a tendency to experience shorter survival times, suggesting that the survival was more associated with the gene status than with the protein levels.

Conclusion

We conclude that most of the NSCLC tumors have high EGFR expression, especially in the SCC subtypes. There was a correlation between increased EGFR gene copy number per cell and protein expression, but a complex interaction between gene and protein levels seems to occur. Increased understanding about molecular characteristics coupled with more comprehensive data on the EGFR cognate ligands EGF and transforming growth factor alpha, and the heterodimerization partners HER-2, HER-3, and HER-4, may provide a better prognostic indicator of response to therapy with EGFR inhibitors.

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