Original Research

Clinicopathologic Characteristics of *EGFR* Mutations in Non-Small Cell Lung Cancer (NSCLC) in Central New York

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EGFR mutation rates in NSCLC differ among ethnic groups, nations, age, sex, smoking status, and histologic differentiation. We examined the clinicopathologic characteristics of EGFR mutations in NSCLC in the Central New York (CNY) region for the first time, to further characterize the disease and facilitate management. 7.1% (33/464) NSCLCs were positive for EGFR mutations. Based on available clinical data, EGFR positive mutation status was found in 7.4% (28/380) White, 5.3% (2/38) African American, 0% (0/4) American Indian/Alaska native, 33.3% (1/3) Asian/Pacific, 0% (0/2) Hispanic, and 0% (0/1) Asian/Indian patients. Average age in EGFR positive cases was 68.3 years and 66 in negative cases (p>0.05). Prevalence of EGFR mutations was 9.2% in females and 4.4% in males (p<0.05); 37.9% in never-smokers and 4.4% in ever-smokers (p<0.05). Poorly differentiated adenocarcinoma was seen in 16.7% (4/24) of EGFR positive cases and 43.4% (105/242) of negative cases (p < 0.05). Solid pattern was seen in 9.0% (2/22) of positive cases and 33.3% (44/132) of negative cases (p < 0.05). The acinar pattern was the most common pattern seen in adenocarcinomas with exon 19 deletion, and exon 21 (L858R) mutations. Therascreen detected four additional rare mutations (1 T790M, 1 L861Q, G719X, and 1 exon 21 insertion) in 238 cases, increasing overall EGFR detection rate from 6.3% to 7.1%. EGFR mutation prevalence in NSCLC in CNY (7.1%) was lower than the global average in a recent review (13.9% [10.3-36.4]), likely due to predominantly white population in this study, accounting for 84.8% (28/33) of positive cases in CNY. Similar to previous studies, positive EGFR mutation status was more frequent in females and never-smokers, and less prevalent in adenocarcinoma with poor differentiation or solid growth.

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INTRUDUCTION

Lung cancer has been traditionally separated into small-cell lung cancers and non-small cell lung cancers (NSCLCs). Lung cancer is the leading cause of cancer related death in both men and women. Although most cases of lung carcinoma are attributable to cigarette smoking, roughly 10 to 40% of lung cancers occur in patients who are never-smokers, this is defined as smoking less than 100 cigarettes in their lifetime.[1-3] The proportion of non-smokers in Asia is higher compared to other countries.[4] Factors that may attribute to cancer in this sub-population may be environmental and/or genetic.[5,6]

Epidermal growth factor receptor (EGFR) tyrosine kinase (TK) domain mutations as well as *EMAP-like protein 4 (EML4)-anaplastic lymphoma kinase (ALK)* fusion are found more commonly in never smokers[4,7] A review of *EGFR* mutation prevalence in lung adenocarcinoma demonstrated 45% of Asian/Pacific, 24% White, 20% African American, 17% Hispanic, and 52% Asian/Indian with *EGFR* mutations.[8] *EGFR* mutations were more common in those younger than 65 (46% vs. 38%), females (58% vs. 32%), and never smokers (58% vs. 26%).[8] *EGFR* mutations occurred in 65% of well-differentiated tumors, 48% of moderately differentiated tumors, and 34% of poorly differentiated tumors.[8]

EGFR mutations have been identified more frequently in mixed acinar and lepidic, followed by mixed papillary and acinar, mixed solid and acinar, mixed micropapillary and acinar, and mixed acinar and mucinous lepidic.[9] In addition, it was found that lesions with mucinous lepidic or papillary patterns more frequently contained mutations compared to lesions with mucinous lepidic or solid components.[9] *EGFR* mutations are seen in all grades of adenocarcinoma, and testing should be performed regardless of adenocarcinoma subtype.[8] It has been shown that solid subtypes present at metastatic locations are associated with shorter overall survival in those

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receiving systemic treatment, and is less likely to possess *EGFR* mutations as compared to micropapillary and acinar patterns.[10] In contrast, patients with resected stage III or IV disease with solid predominant tumors showed greater benefit of postoperative chemotherapy and/or radiation compared with non-solid types.[11] When comparing histologic patterns in metastatic sites to primary sites, solid and micropapillary are more likely to be seen at metastatic sites as compared to the percentage seen at the primary site.[12,13] Solid histology and signet ring cells are seen more often in *ALK* rearranged tumors in Western populations, however not as commonly in Asian populations.[14]

With this information, we reviewed lung cancer cases in the Upstate New York region including histology, smoking status, and ethnicity, and analyzed the clinicopathologic correlation with status of EGFR gene mutations to facilitate the patient management in this region.

METHODS

NSCLC cases (n=464) with available molecular and clinical data were selected from the Upstate Pathology Molecular lab (2014-2016). Thirteen cases were excluded due to accessioning errors. One case failed because the specimen was decalcified. Seven cases were excluded because of insufficient tumor. This study was granted exemption from the institutional review board of our institute. 23 cases were resections, 314 biopsies, and 127 cases were from cell blocks**.** Clinical data was extracted through the EMR. Never-smokers were defined as those patients who smoked <100 cigarettes in their lifetime.

Molecular testing was previously run on paraffin embedded tissues by either therascreen *EGFR* RGQ PCR kit covering exon 18, 19, 20, and 21 mutations (n = 238) or a lab-developed test (LDT) covering only exon 19 deletion and L858R point mutation (n = 226). LDT was used if therascreen failed or if too few samples were obtained per week. Histological patterns were only reviewed in core biopsy or resection specimens that were available, and cell block specimens were not assessed for histological pattern.

Molecular testing was run on paraffin embedded tissues by either therascreen *EGFR* RGQ PCR kit covering exon 18, 19, 20, and 21 mutations (n = 238) or a lab-developed test (LDT) covering only exon 19 deletion and L858R point mutation (n=226). LDT was used if therascreen failed or if too few samples were obtained per week. Therascreen EGFR RGQ PCR Kit (Qiagen, Valencia, CA) was performed on the Rotor-Gene Q MDx instrument. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue. Tumor areas were identified by the attending pathologist who signed out the case, and tumor areas were manually microdissected. The LDT was performed on paraffin embedded tissues with two separate assays. One for detection of deletions at exon 19, and the other for point mutations at exon 21. Exon 19 deletions were present if any PCR product, ranging from 183bp to 198 bp was identified. An exon 21-point mutation was defined as an 87 bp restriction digest product of the amplicon. A Sau961 restriction enzyme is used to create a new restriction site. A minimum of 500 tumor cells was usually required for this assay.

The presence of different adenocarcinoma patterns, including acinar, papillary, micropapillary, lepidic, solid were defined using definitions from the WHO Classification of the Lung,[15] was recorded as a binary variable. In each case one or two major histologic patterns were identified, and those with more than one pattern were noted as combined patterns. Focal patterns (less than or equal to 5%) were also identified. Cell blocks from fine needle aspirations were not included in pattern analysis.

A z-test was performed to compare patient characteristics, histologic diagnoses, and molecular data. The characteristics were compared using Social Science Statistics website (www.socscistatistics.com). In all tests, a p value of < 0.05 was considered a significant difference between the two compared sets of data.

RESULTS

7.1% (33/464) NSCLCs were positive for *EGFR* mutations. Based on available clinical data, EGFR positive mutation status was found in 7.4% (28/380) White, 5.3% (2/38) African American, 0% (0/4) American Indian/Alaska native, 33.3% (1/3) Asian/Pacific, 0% (0/2) Hispanic, and 0% (0/1) Asian/Indian patients. Average age in *EGFR* positive cases was 68.3 years and 66 in negative cases ($p > 0.05$). Out of the total number of patients in the study, 386 patients had a history of smoking while 38 patients were never-smokers. Prevalence of *EGFR* mutations was 9.2% in females and 4.4% in males ($p < 0.05$); 37.9% amongst never-smokers and 4.4% in ever-smokers ($p < 0.05$). Poorly differentiated adenocarcinoma was seen in 16.7% (4/24) of *EGFR* positive cases and 43.4% (105/242) of negative cases ($p < 0.05$). Solid pattern was seen in 9.1% (2/22) of positive cases and 33.3% (44/132) of negative cases ($p < 0.05$) (**Figure 1**). 50% (6/12) of cases with exon 19 deletions, and 50% (4/8) of cases with exon 21 mutations (L858R) showed acinar pattern, however was not statistically significantly different ($p > 0.05$). One case with exon 18 G719X mutation showed a lepidic pattern. One case with exon 21 (L861Q) mutation showed a solid pattern. Two major mutations, exon 19 deletions and exon 21 L858R, were detected in 6.3%. Therascreen detected four additional rare mutations (1 T790M, 1 L861Q, G719X, and 1 exon 21 insertion) in 238 cases, increasing overall EGFR detection rate 0.8% (**Figure 2**).

The site of tissue sampling included lung (316), lymph node (63), bone (22), pleural fluid (15), soft tissue (12), liver (12), brain (5), pleura (5), pericardial fluid (3), mediastinum (3), adrenal (3), and one each for abdomen, cerebrospinal fluid, spleen. Median progression free survival was 12 months [7-24], which is comparable to historical data, and median overall survival was not reached for patients who received a TKI (n = 10). Histology of selected *EGFR* positive cases (**Figure 3A-3D**).

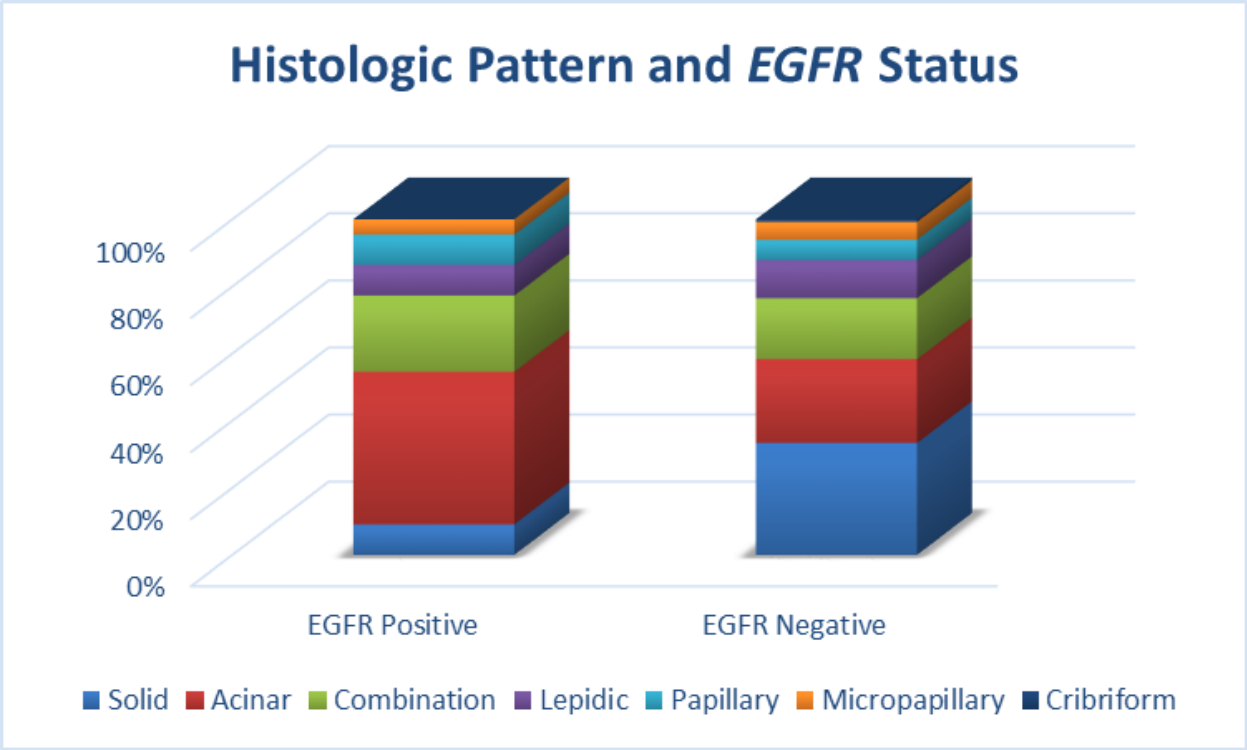

Figure 1. Different histologic patterns seen in *EGFR* positive cases.

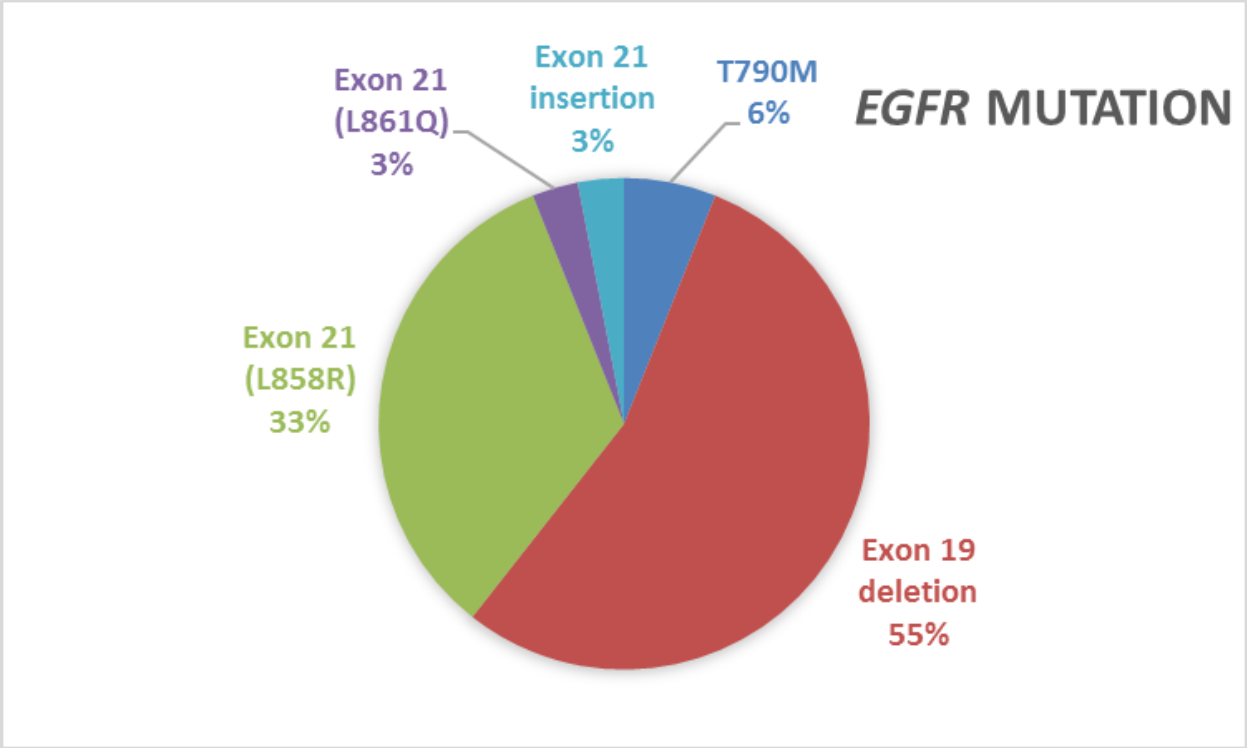

Figure 2. *EGFR* mutation type found in positive cases.

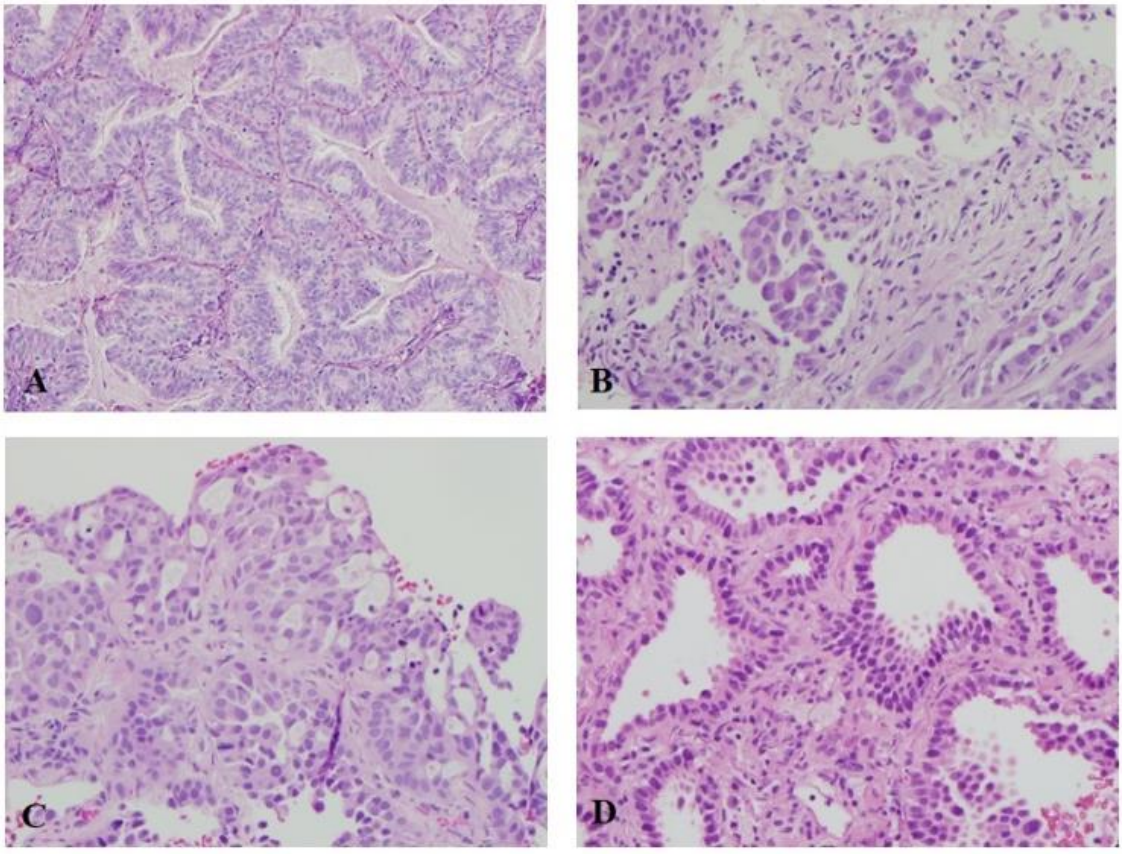

Figure 3. Moderately differentiated adenocarcinoma from wedge resection showing papillary architecture with Exon 19 *EGFR* deletion, 10x (**A**). Poorly differentiated adenocarcinoma from transbronchial biopsy showing micropapillary and cribriform patterns which were positive for exon 19 *EGFR* deletion, 20x (**B, C**). Well-differentiated adenocarcinoma from core needle biopsy showing acinar pattern which was positive for exon 19 *EGFR* deletion, 20x (**D**).

DISCUSSION

EGFR mutation prevalence in NSCLC in CNY (7.1%) was lower than the global average (13.9% [10.3-36.4]), likely due to predominantly white population in this study, accounting for 84.9% (28/33) of positive cases in CNY. Although overall *EGFR* mutation prevalence was lower in CNY, most clinicopathologic characteristics in this region were similar to previous studies in other areas, i.e. positive *EGFR* mutation status was more frequent in females and never-smokers and less prevalent in adenocarcinoma with poor differentiation or solid growth. Most of the patients in this study had a history of smoking (91%). The prevalence of smoking in Central New York was 22.9% when surveyed from 2013-2014.[16] This is higher than the national prevalence of smoking in the United States at 15.1% in 2015.[17] The higher prevalence of smoking may contribute to the decreased detection of *EGFR* mutations in this study. Mutations seen in smokers with lung cancer include *p53*, *CDKN2* and *FHIF*, rather than *EGFR* mutations.[18] The rate of lung cancer in New York State does not seem to be higher as compared to other states from 1999 to 2013.[19] Interestingly, *EGFR* mutations tend to be seen in older patients, on average, 68.3 year old in this study. This is similar to previous observations in an Asian population, although the average age of cancer diagnosis was younger in that study.[20]

In a study, which analyzed exon 19 deletions, and exon 21 L858R mutations, by Paik at Memorial Sloan Kettering the prevalence of *EGFR* mutations was 37% in never-smokers and 14% in former/current smokers ($p < .0001$).[21] Median age was 63 in never smokers and 68 in former/current smokers that were *EGFR* positive, while those uncharacterized were 66 in never smokers and current/former smokers. 134/164 positive *EGFR* patients were white with an *EGFR* mutation prevalence of 24.3% (164/675). An earlier study by D'Angelo demonstrated a prevalence of 23.5% (503/2142), however ethnicity was not assessed.[22] Prevalence of *EGFR* mutation studied in other countries include 9.8% (118/1201) in Germany, 16.7% (121/753) and 10.3% (1047/10117) in France, 36.4% (112/308) in Japan, 16.6% (350/2105) in Spain, 12.7% (52/411) in Italy.[23-28]

More recent oncogenes that have been found in lung adenocarcinoma include *human epidermal growth factor receptor 2 (HER2)* insertions, *BRAF* mutations,

phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (*PIK3CA)* mutations, *RET* and, *ROS1* rearrangements.[29] One study identified HER2 mutations in 1.6% (11/671) NSCLCs, and were more often seen in never smokers (3.2%), and those with adenocarcinoma histology (2.8%).[30] Out of a total of 394 cases of adenocarcinoma it was seen in 3.9% of Asians, 0.7% non-asian ethnicities, 3.6% of females, 1.9% of males, 4.1% of never smokers, and 1.4% of smokers.[30] Studies looking at *BRAF* missense mutations in adenocarcinoma of the lung found a rate of 3% (n = 697), and 4.9%.[31,32] *PIK3CA* mutations have been seen at a slightly lower rate between 1.3 and 3.4% of NSCLC.[29]

In conclusion, the prevalence of *EGFR* mutations in CNY was lower than the global average, most likely due to the higher White population and increased smokers in this region. This study also implies that many other mutations other than *EGFR*, are contributing to lung carcinogenesis in this region. Therefore, extensive and profound investigation of other genetic alterations is becoming more important in management of lung cancer in this region. Next generation sequencing, i.e. massive parallel sequencing, will become an efficient and indispensable new tool to accelerate this substantial mission.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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