Correlation of Cytomorphology and Molecular Findings in EGFR+, KRAS+ and ALK+ Lung Carcinomas

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Abstract

Lung carcinoma is one of the leading causes of cancer deaths worldwide for both men and women. Established risk factors & probable risk factors, which are implicated in the occurrence of lung carcinomas have been identified and evidently present in the populace, especially in our Indian scenario, e.g., tobacco chewing, smoking & many more. With the increase in the incidence & prevalence of lung carcinomas, correct diagnosis at an appropriate time, has become a necessity. Cytomorphology has been in vogue since long and is still one of the most relied upon methods. Contemporarily, in the past few years, there has been a dramatic increase in what we know about the molecular genetic features of this disease entity & its various diagnostic applications. With this new knowledge, there have been new targeted non-chemotherapeutic agents available to the patients, which have shown variable efficacy in tumors of different histologic subtypes and molecular status. The correlation of the cellular features & the molecular status of these carcinomas can well be established as a synergistic tool for effective diagnosis & targeted therapy.

Key Words : ALK (Anaplastic Lymphoma Kinase), EGFR(Epidermal Growth Factor Receptor), KRAS (Kirsten Rat Sarcoma viral oncogene),Lung carcinoma.

Introduction

Lung cancer has varied epidemiology depending on the geographic region. Globally, there have been important changes in incidence trends amongst men and women and incidence in non-smokers. Worldwide, there are 1.61 million new cases of lung cancer per year, with 1.38 million deaths, making lung cancer the leading cause of cancer-related mortality. [1] In India, approximately 63,000 new lung cancer cases are reported each year. [1] For the therapeutic and prognostic purpose, lung carcinomas are classified into small cell carcinomas, non small cell carcinomas and mixed carcinomas. These tumours can be further classified into several major histological subtypes, including adenocarcinoma, squamous cell carcinoma, large cell carcinoma, adenosquamous cell carcinoma, and sarcomatoid carcinoma.[2] The immense scientific advances made in the past decade have facilitated the in depth characterization of different disease subtypes, based on their genetic profiles. This has profound implications in certain types especially non small cell lung cancer (NSCLC) which is the commonest cause of cancer deaths worldwide. [3] Histologic studies also have suggested some peculiar morphologic features predominating in tumours. [4]

Over the last four decades, there has been a shift in the pathologic distribution of NSCLC. Before 1970s, squamous-cell carcinoma was the most common histological type of NSCLC, but since about 1975, there has been a dramatic increase in the incidence of adenocarcinoma, making it the predominant histological subtype of NSCLC. [1] A review article from 2004 stated that squamous-cell carcinoma was still the predominant histological subtype of NSCLC in India. In another study, adenocarcinoma accounted for 44% of NSCLC, while only 26% were squamous-cell carcinoma. Results from similar studies suggest that a pathologic shift may have occurred in India as well. [1]

Study done by Noronha et al shows that 8% of patients had small-cell carcinoma. Of the 92% patients with non-small-cell carcinoma (NSCLC), the most common histology was adenocarcinoma (43.8%), followed by squamous cell carcinoma (26.2%), large cell carcinoma (2.1%) and other (8.3%). [1]

EGFR (Epidermal Growth Factor Receptor), is located at chromosome 7p11.2, spans about 200kb, and contains 28 exons. [2] It is the cell surface receptor for Epidermal Growth Factor family of extracellular protein ligands. EGFR signalling is not only critical for cell proliferation, but also to processes that are crucial for cancer

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progression, including angiogenesis, metastasis, and inhibition of apoptosis. The tyrosine kinase (TK) is the part of this protein located inside the cell, which switches on when a growth factor or ligand from outside of the cell binds to the outside protein of the EGFR. This switch when turned on allows the EGFR to signal the cells to grow and survive. [5] It is this tyrosine kinase which is targeted for therapy in EGFR positive carcinomas with Tyrosine Kinase inhibitors (TKI). [2]

In adenocarcinomas, the majority of mutations have been identified in exons 18 21 of the EGFR gene. [2] Current data indicate that EGFR mutations are adenocarcinoma dominant, rather than adenocarcinoma specific. Clinically, most EGFR mutations are detected in adenocarcinomas; with other types of lung carcinomas showing a much lower frequency of EGFR mutations: 5% in squamous cell carcinomas and virtually none in large cell carcinomas. [2] Adenosquamous carcinomas appear to have an EGFR mutation incidence that is similar to that of adenocarcinomas.

The KRAS (Kirsten Rat Sarcoma) protooncogene encodes KRAS G-protein, which has a critical role in the RAS/MAPK1 signaling pathway downstream of many growth factor receptors, including EGFR. RAS genes encode a family of membrane bound 21 KD GTP binding proteins that regulate cell growth, differentiation, and apoptosis by interacting with multiple effectors including those in the MAPK (Mitogen Activated Protein Kinase), STAT (Signal Transducer and Activator of Transcription) and PI3K (Phosphatidylinositol 3 Kinase) signaling cascades. RAS proteins acquire oncogenic potential when amino acid at positions 12, 13, or 61 is replaced as a result of a point mutation in the gene. This point mutation leads to constitutive activation of RAS signaling pathway. RAS mutations are prevalent in all human malignancies, but of them, KRAS is the most common. KRAS accounts for 90% of RAS mutations in lung adenocarcinoma, and 97% of KRAS mutations in NSCLC involve codon 12 or 13. KRAS mutations are uncommon in squamous cell carcinoma of the lung. [5]

ALK/EML4 (Anaplastic Lymphoma Kinase/ Echinoderm Microtubule Associated protein like) fusion results from an inversion in short arm of chromosome 2 that juxtaposes the EML4 gene with the ALK gene. ALK/EML4 fusion results in protein oligomerisation and constitutive activation of the kinase. [5]

Molecular studies can be adequately tested on cytologic specimens, including fine needle aspiration (FNA) and fluid cell block sections. Furthermore, small biopsies, such as endobronchial ultrasound-guided FNA, are increasingly being performed on patients. [4]

EGFR, KRAS and ALK mutation analysis

The most commonly used method to detect mutations is direct sequencing. It is a vital observation that tissue slides many-a-times contain heterogeneous components of cells, a fact that at times hampers optimal analysis & proper diagnosis. In addition, some patients present with multifocal lung tumors. Careful dissection of cells from a suitable representative area is pivotal to ensure a successful test result.[2] EGFR and KRAS mutation analysis is done by extraction of DNA, performed from the cytology fluid or a manually microdissected cell block sample provided. For the detection of mutations, DNA is amplified with primers flanking exon 18, exon 19, exon 20 and exon 21 of the EGFR gene; and exon 2 and exon 3 of the KRAS gene. Then, polymerase chain reaction products are sequenced in both sense and antisense directions and the sequences are analyzed. Each case is classified as positive or negative for the EGFR and KRAS mutation based on the sequencing results. EML4-ALK fusion gene is analyzed by fluorescence in situ hybridization; most commonly performed method. [4]

Other methods include PCR single-strand conformational polymorphism analysis and high resolution-melting amplicon analysis. Relative to the direct sequencing method, the other two techniques allow for the rapid detection of mutations with high sensitivity and specificity. However, confirmation of mutations via direct sequencing is still necessary. Though not of any current clinical use, an assay that provides a rapid assessment of EGFR mutation status in as little as 30 minutes using a 'smart amplification process' has been described. These may one day provide greatly improved turnaround times for this analysis. Formalin-fixed and paraffin-embedded tissue is perfectly suitable for fluorescence in situ hybridization (FISH) and DNA-based tests, but tissue preservation is critical for a successful test. Decalcified and ethanol-fixed tissue, as well as tissues containing abundant necrosis, should be avoided. [2]

Table 1: Comparison of different studies showing percentage positivity in EGFR, KRAS and ALK gene mutation in lung carcinomas:

Study author	EGFR	KRAS	ALK
Zaibo Li et al [4]	14%	31%	3%
Shi Y et al [6]	51.4%	-	-
Noronha et al [3]	35%	-	-
Rasmita Sahoo et al [7]	51.8%	-	-
Chouqhule A et al [8]	23%	-	-
Xia N et al [9]	52.7%	3.6%	-
Li Y et al [10]	24.5%	2.88%	3.37%
Wanq J et al [11]	44.9%	7.2%	9.6%
Smits AJ et al[12]	9.1%	33.3%	-
Bae NC et al [13]	17.4%	5.2%	-
Jong Mu sun et al [14]	38%	8%	-
Subramanian et al [15]	17%	22%	7%
Christian Boch et al [16]	4.9%	15%	-
Janq TW et al [17]	24%	9.6%	-
Jianya Zhou et al [18]	52.2%	-	8.4%
Shigematsu et al [19]	21%	8%	-

Table 2: Comparison of cytomorphologic features with EGFR, KRAS, ALK mutation positive lung carcinoma: [4]

Cytomorphologic features [4]	EGFR	KRAS	ALK
Growth pattern	Glandular, papillary, or mixed glandular -papillary growth patterns.	Poorly differentiated growth pattern without acinar or papillary architecture	Acinar or glandular growth pattern.
Mean nuclear grade	1.9	2.2	2.3
Eosinophilic granular cytoplasm	6%	32%	100%
Necrosis	12%	65%	67%

Discussion

Testing of EGFR and KRAS mutations is now a common practice among community oncologists, and more recently, ALK rearrangements have been added to this group. [5] From the comparison of different studies as shown in table-1; this variation in the frequency of EGFR, KRAS and ALK gene mutation observed around the world may be because of environmental, geographical and ethnic differences and different methodologies of mutation detection. From table-2, it can be seen that cytologic features of ALK+ and KRAS+ tumors included more nuclear pleomorphism, less vacuolated cytoplasm and necrosis than did EGFR+ tumors, which may explain the less definitive subclassification in ALK+and KRAS+ tumors.

Conclusion

EGFR has been found to be the commonest gene mutation associated with lung carcinomas worldwide. Its identification has become an integral part of the diagnostic arrays for lung carcinomas. Furthermore, the association of adenocarcinomas with EGFR positivity is noteworthy. This also implies that the patients with adenocarcinomas may have increased chances of EGFR positivity & hence, may probably be benefitted by the tyrosine kinase inhibitors. This correlation between the cytomorphology & genetic status of the tumor can prove helpful for targeted therapy in the patients of lung carcinomas.

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