Case Report

Increased Copy Number of Anaplastic Lymphoma Kinase Gene Signal in Lung Carcinomas: Is it Significant?

Saumya Shukla, Nuzhat Husain, Kiran Preet Malhotra, Vandana Tiwari[1]

Departments of Pathology and [1]Biochemistry, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Abstract

The anaplastic lymphoma kinase gene re-arrangement which is present in 3-5% cases of non small cell lung cancer is a somatic gene re-arrangement. The gold standard for the identification of this gene re-arrangement is fluorescence in situ hybridization. Many variant hybridization patterns have been documented. We present a case of polysomy of ALK gene in the absence of ALK gene re-arrangement in a 45 year old female who presented with brain metastasis. This is a rare case of polysomy of ALK gene reported in a non small cell lung carcinoma. It may be indicative of a worse prognosis and may predict high metastatic potential in these tumors.

Keywords: Anaplastic lymphoma kinase, fluorescence *in situ* hybridization, polysomy

Introduction

Anaplastic lymphoma kinase (ALK) gene rearrangements were first reported in nonsmall cell lung cancer (NSCLC) in the year 2007. The novel ALK fusion with echinoderm microtubule-associated protein-like 4 (EML4-ALK) gene was first discovered as a somatic gene rearrangement by Hiroyuki Mano *et al*. An inversion event on the short arm of chromosome 2, resulting in the fusion of ALK gene with the EML4 gene locus, is the most common aberration of the ALK gene in lung cancer This rearrangement leads to the production of a chimeric protein, which has constitutive ALK kinase activity, resulting in the inhibition of apoptosis and promotion of cell proliferation in tumor cells. The ALK gene rearrangement is found in 3%–5% of cases of NSCLC.[1-4]

A series of clinical and pathological features have been documented in patients who harbor this translocation. This alteration is most frequently detected in younger patients and the most common histologic pattern is the solid or signet ring pattern. It is most frequently detected in nonsmokers and is associated with hepatic, brain metastasis, and pleural and pericardial effusions. No apparent differences in the ethnicity and sex have been identified.[4,5]

Received: 09.03.2019 **Revised:** 10.04.2019
Accepted: 21.04.2019 **Published:** 18.11.2019

Access this article online

Quick Response Code:

Website: http://www.jmau.org/

DOI: 10.4103/JMAU.JMAU_9_19

Crizotinib is an oral selective inhibitor of ALK and mesenchymal–epithelial growth factor (c-Met)/hepatocyte growth factor kinases. Based on the response rates reported in the Phase 1 and 2 clinical trials, crizotinib received accelerated approval by the Food and Drug Administration in August 2011 for the treatment of locally advanced or metastatic NSCLC that show evidence of ALK gene rearrangement.[5,6]

There are various methods for the detection of ALK gene rearrangement in NSCLC; however, the gold standard is fluorescence *in situ* hybridization (FISH) performed on formalin-fixed paraffin-embedded tissue blocks.[1] The analysis of the ALK gene rearrangement involves the assessment of the integrity of the gene. The commercial assay contains a spectrum orange labeled 300-kb probe on the telomeric 3' side of ALK and a spectrum green labeled 442-kb probe on the centromeric 5' side. The wild-type configuration is seen as a fused yellow signal. The cells are considered positive for the ALK gene rearrangement when the adjacent red and green signals are more than two signal diameter apart and/or a fused

Address for correspondence: Dr. Saumya Shukla, Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow - 226 010, Uttar Pradesh, India.
E-mail: saumyavarun@gmail.com

How to cite this article: Shukla S, Husain N, Malhotra KP, Tiwari V. Increased copy number of anaplastic lymphoma kinase gene signal in lung carcinomas: Is it significant? J Microsc Ultrastruct 2019;7:198-200.

signal exists with a single red signal. A sample is considered negative when <10% of the tumor cells show evidence of ALK gene rearrangement. A sample is considered distinctly positive when >50% of cells show the rearrangement. A sample is considered as equivocal if 10%–50% of cells show the rearrangement.[4-6]

Few variant hybridization patterns besides rearrangement are known to occur rarely.[6] We present this case with a variant hybridization pattern of the ALK gene in the absence of ALK rearrangement.

Case Report

A 45-year-old female presented with a history of headache and seizures associated with loss of consciousness. The magnetic resonance imaging of the brain revealed solid large-enhancing lesion in the frontal lobe of the brain. Craniotomy revealed a large tumor infiltrating diffusely into the cerebral parenchyma. A biopsy was taken, and the histopathological examination revealed a tumor composed of neoplastic cells arranged in acini with focal papillary configuration. The cells had moderate amount of amphophilic cytoplasm with the vesicular nucleus and prominent nucleoli. Mitosis along with necrosis was also appreciable [Figure 1]. The histomorphology was in favor of metastatic adenocarcinoma. An immunohistochemical panel was performed to identify the site of the primary tumor. The tumor was diffusely positive for cytokeratin 7, with focal expression of thyroid transcription factor-1 and negative for cytokeratin 20. Glial fibrillary acidic protein highlighted the glial tissue infiltrated by tumor [Figure 2].

Based on the morphology and immunohistochemical marker expression, a diagnosis of metastasis from pulmonary adenocarcinoma was rendered.

FISH for detection of the ALK gene rearrangement using the Vysis ALK Break Apart Probe Kit (Abbott molecular) was performed. The analysis of the ALK gene revealed that 2% of the nuclei had the break apart signal and most of the nuclei had a fused red and green signal (yellow color). About 25%–30% of nuclei had four or more ALK gene copies which were in indicative of low-grade polysomy of ALK without ALK rearrangement [Figure 3].

On further workup, the patient was detected to have a small nodular lesion in the right lung on computed tomography scan, which was accepted as the silent primary tumor leading to brain metastasis. The patient fared poorly after diagnosis and succumbed to intracranial tumor bleed a fortnight later.

Discussion

ALK is a transmembrane protein, a member of the insulin-like tyrosine kinase receptor superfamily. Various combinations of ALK with other proteins have been discovered in other neoplasms such as diffuse large B-cell lymphoma, inflammatory myofibroblastic tumor, neuroblastoma, squamous cell carcinoma of the esophagus, and renal cell carcinoma.[6,7]

An increased copy number of fused nonrearranged ALK signals corresponds to polysomy (≥4 ALK copies in ≥10% nuclei) or ALK amplification (≥10 ALK copies in ≥10% nuclei). An increase of ALK gene copy number observed in 10%–39% of cell nuclei is considered as low-grade polysomy, whereas >40% of cell nuclei is considered as high-grade polysomy. This division has been adapted from the FISH test results scale as used by Sasaki *et al*. in their study for evaluation of copy number abnormalities NSCLC patients.[7]

The clinical significance of ALK gene amplification and increased copy number of ALK is not known.[7,8] Wojas-

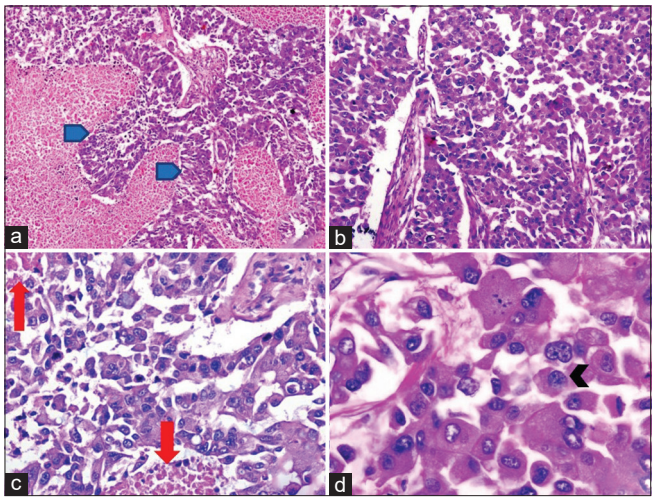

Figure 1: (a) Tumor composed of neoplastic cells in the papillary and sheet-like pattern (blue arrowhead) with intervening necrosis (H and E, ×50). (b and c) Tumor cells with high nucleo-cytoplasmic ratio and moderate pleomorphism and necrosis (red arrow) (b: H and E, ×100, c: H and E, ×200). (d) Tumor cells with a vesicular nucleus and prominent nucleoli (black arrowhead) (H and E, ×400)

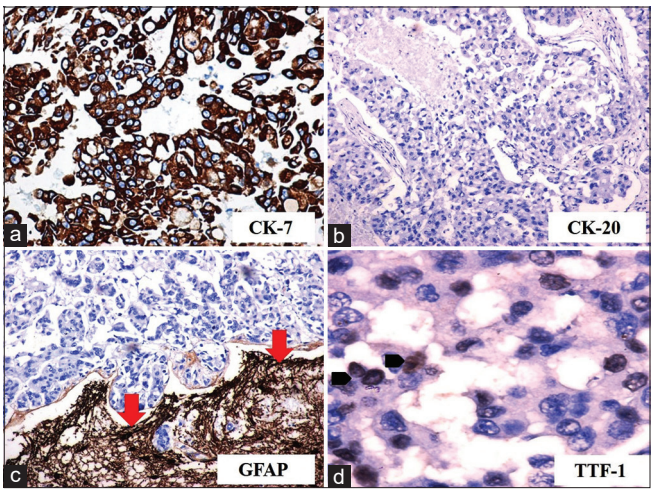

Figure 2: (a) Cytokeratin 7: Diffuse cytoplasmic-positive staining in tumor cells (DAB, ×200). (b) Cytokeratin 20: Negative staining in tumor cells (DAB, ×200). (c) Glial fibrillary acidic protein: Negative in the tumor cells, positive in the glial tissue (red arrow) (DAB, ×200). (d) Thyroid transcription factor-1: Focal nuclear-positive expression in tumor cells (black arrows) (DAB, ×200)

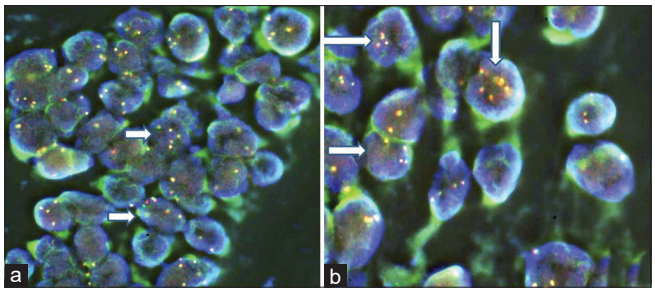

Figure 3: (a and b) Fluorescent *in situ* hybridization for anaplastic lymphoma kinase gene rearrangement showing fused red and green signals (yellow color). 25%–30% of the nuclei had ≥4 anaplastic lymphoma kinase gene copies (white arrows) indicative of low-grade polysomy (oil, ×1000). (DAPI/blue color stains cellular nuclei)

Krawczyk *et al*. observed that ALK gene amplification in NSCLC was associated with the past or present history of smoking, whereas polysomy had no association with smoking. Hence, this indicates that ALK gene amplification could be involved in carcinogenesis of lung cancer associated with smoking.[5] However, the prognostic value of this amplification was not evaluated.

This association between increased ALK gene copy number and prognosis needs further evaluation. Several case series on amplification of other genes involved in lung carcinogenesis have documented their prognostic significance. EGFR protein overexpression and gene amplification in NSCLC are associated with aggressive tumor behavior.[6] Sasaki *et al*. stated that patients of NSCLC with KRAS polysomy or amplification showed significantly worse prognosis, when compared with patients who harbored disomy of KRAS.[7] A high mesenchymal–epithelial transition (MET) factor gene copy number is indicative of shorter survival in patients of NSCLC.[8]

In our case, the patient presented with brain metastasis with unknown primary and had a short survival. Hence, polysomy and amplification of the ALK gene may be indicative of worse prognosis and poor survival in lung carcinomas as well.

Desai *et al*. and Dai *et al*. stated the polysomy and ALK amplification signify the genetic differences in tumor tissue. The amplification of the ALK fusion gene is reported in a small number of crizotinib-resistant tumors, with or without concurrent ALK mutations.[1,9-11] In our case, response to crizotinib could not be evaluated due to poor survival, but this association of probable drug resistance with polysomy also needs further evaluation.

Typical and variant hybridization patterns for ALK gene rearrangements are likely due to intratumoral heterogeneity. The presence of an increased copy number of the ALK gene must be documented, as it may be indicative of poor prognosis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Acknowledgement

Pfizer Oncology India Pvt. Ltd. for routine screening of ALK in cases of NSCLC.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Desai SS, Shah AS, Prabhash K, Jambhekar NA. A year of anaplastic large cell kinase testing for lung carcinoma: Pathological and technical perspectives. Indian J Cancer 2013;50:80-6.
2. Park HS, Lee JK, Kim DW, Kulig K, Kim TM, Lee SH, *et al.* Immunohistochemical screening for anaplastic lymphoma kinase (ALK) rearrangement in advanced non-small cell lung cancer patients. Lung Cancer 2012;77:288-92.
3. McLeer-Florin A, Moro-Sibilot D, Melis A, Salameire D, Lefebvre C, Ceccaldi F, *et al.* Dual IHC and FISH testing for ALK gene rearrangement in lung adenocarcinomas in a routine practice: A French study. J Thorac Oncol 2012;7:348-54.
4. Pilotto S, Peretti U, Novello S, Rossi G, Milella M, Giaj Levra M, *et al.* PROFILing non-small-cell lung cancer patients for treatment with crizotinib according to anaplastic lymphoma kinase abnormalities: Translating science into medicine. Expert Opin Pharmacother 2013;14:597-608.
5. Wojas-Krawczyk K, Krawczyk PA, Ramlau RA, Szumiło J, Kozielski J, Kalinka-Warzocha E, *et al.* The analysis of ALK gene rearrangement by fluorescence *in situ* hybridization in non-small cell lung cancer patients. Contemp Oncol (Pozn) 2013;17:484-92.
6. Suzuki S, Dobashi Y, Sakurai H, Nishikawa K, Hanawa M, Ooi A. Protein overexpression and gene amplification of epidermal growth factor receptor in nonsmall cell lung carcinomas. An immunohistochemical and fluorescence *in situ* hybridization study. Cancer 2005;103:1265-73.
7. Sasaki H, Hikosaka Y, Kawano O, Moriyama S, Yano M, Fujii Y. Evaluation of kras gene mutation and copy number gain in non-small cell lung cancer. J Thorac Oncol 2011;6:15-20.
8. Go H, Jeon YK, Park HJ, Sung SW, Seo JW, Chung DH. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol 2010;5:305-13.
9. Salido M, Pijuan L, Martínez-Avilés L, Galván AB, Cañadas I, Rovira A, *et al.* Increased ALK gene copy number and amplification are frequent in non-small cell lung cancer. J Thorac Oncol 2011;6:21-7.
10. Dai Z, Kelly JC, Meloni-Ehrig A, Slovak ML, Boles D, Christacos NC, *et al.* Incidence and patterns of ALK FISH abnormalities seen in a large unselected series of lung carcinomas. Mol Cytogenet 2012;5:44.
11. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, *et al.* Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012;18:1472-82.