Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 4S (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (Rosi) genes on lung cancer samples of Iraqi patients

Mays Talib Abdallah[*1], Mahmoud H. Khalaf Al-Fahdawi[1], Zaid Mohammed Joodi Al Janabi[1], Mohammed Essam Abdulmuttaleb[2], Ahmed Zuhair Alsammarraie[3], Noor Jabbar Dawood [1], Mohammed Kadhom[4]

[1]Collage of biotechnology, Al-Nahrain University, Baghdad, Iraq

[2]General Heet Hospital, Al-Anbar City, Iraq

[3]Oncology Teaching Hospital, Baghdad Medical City, Iraq

[4]Deprtmant of Environmental Health, College of Energy and Environmental Science, Alkarkh University of Science, Baghdad, 10081, Iraq

***Corresponding Author**

Email ID: mays.talip@nahrainuniv.edu.iq

Cite this paper as: Mays Talib Abdallah, Mahmoud H. Khalaf Al-Fahdawi, Zaid Mohammed Joodi Al Janabi, Mohammed Essam Abdulmuttaleb, Ahmed Zuhair Alsammarraie, Noor Jabbar Dawood, Mohammed Kadhom, (20xx) Anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (Rosi) genes on lung cancer samples of Iraqi patients. *Journal of Neonatal Surgery*, 14 (4s), 602-607.

ABSTRACT

Lung cancer, a global disease, is classified into two main categories: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is accounted for 85% of all lung cancer diagnoses and is divided into three subgroups: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The most common type is adenocarcinoma which occurs most commonly in non-smokers, and more frequently in females than males. Both genetics and environmental factors play a role in the development of lung cancer. The anaplastic lymphoma kinase (ALK) gene located on chromosome 2 is a key regulator of cell proliferation and signal transduction. In addition to anaplastic large-cell lymphoma (ALCL), ALK gene alterations were initially associated with this malignancy, mutations, and fusions; they have now also been implicated in other malignancies, including non-small cell lung cancer (NSCLC). The way how signaling and differentiation in cells, like neuronal ones, happens also depends on the ROS1 gene located on chromosome 6q22.1.

Other ROS1 alterations such as the ROS1-ALK fusion have also been implicated in the progression of NSCLC. Employing cutting-edge molecular techniques such as targeted RNA sequencing and real-time PCR this study aimed to detect ALK and ROS1 gene fusions in non-small cell lung cancer patients. Our findings indicate that these genetic alterations play a significant role in patients' health and may serve as targets for therapies, particularly TKIs. The study accentuates the advantages of molecular diagnostic methods such as RT-PCR over traditional methods such as fluorescence in situ hybridization (FISH) based on their sensitivity, cost efficiency, and throughput.

Keywords: *Anaplastic lymphoma kinase; c-ros oncogene; lung cancer; NSCLC; ALK gene; Gene fusion*

1. INTRODUCTION

Lung cancer is one of the most frequent and lethal cancers worldwide impacting millions of patients [1]. They are divided into two major types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). These types of cancer have various biological characteristics, treatment and patient prognosis. NSCLC is the most frequent type of lung cancer and surgical resection and targeted therapy can be performed to cure the disease. Meanwhile, SCLC has a more potent and distinct reaction to chemotherapy [2]. However, still present is high unmet need for lung cancer with lung cancer research and therapy advancement. Determining its place as a significant public health concern is of utmost importance and requires constant awareness and investment [3].

The reliability of NSCLC in lung cancer oncology is evident as it accounts for approximately 85%. There are three significant subtypes of this tumor, i.e., adenocarcinoma, large cell carcinoma, and squamous cell cancer [2]. Adenocarcinoma, the most frequent subtype of non-smokers, is typically diagnosed in women and non-smokers. Thus, the changing dynamics of lung cancer come into focus, where environmental and genetic factors may have a significant role to play in its causation [4]. About 15% of lung cancer is small cell lung carcinoma (SCLC), which causes rapid growth and early dissemination of cancer.

It is a smoking-related type of lung cancer and typically diagnosed at late stages [5]. Lung cancer is still a significant public health concern and maybe one of the biggest cancer-related killers worldwide. Its pathogenesis is controlled by various genes, and recent research and clinical practice ever more highlight the anaplastic lymphoma kinaboliclase (ALK) and c-ros oncogene 1 (ROS1) genes. The genes are relevant in that they are targets for targeted therapies acting to improve patient prognosis, acting against specific genetic changes. This advancement is reflective of the increasing importance of precision medicine in transforming the treatment of lung cancer [6].

1.1 ALK gene

ALK is located on chromosome 2 and encodes for a receptor tyrosine kinase with a role of regulating cell growth and signaling [7]. ALK has been implicated in various cancers, including neuroblastoma and lung cancer. ALK was initially found to be associated with anaplastic large-cell lymphoma (ALCL), a tumor associated with a chromosomal translocation [8]. Appreciating its function in other cancers puts into perspective its multifunctional function in cancer development and further enhances its aptness as a therapeutic agent in other cancers [2]. The ALK protein is found as a major receptor that, through the activation of its single ligands, initiates cascading sequences of signals. These pathways, particularly the PI3K-AKT and RAS-MAPK ones, are central to these pathways and play key roles in regulating cellular processes such as proliferation, survival, and differentiation [9,10]

Mutations and rearrangements of the ALK gene have been identified as causative agents in the pathogenesis and progression of numerous cancers [11]. The most important one is the formation of the ALK fusion protein, which occurs due to chromosomal rearrangements. In ALCL and certain subtypes of NSCLC, the ALK gene fuses with other genes, leading to the production of a constitutively active ALK protein. This abnormal protein causes uncontrolled growth and division of cells and contributes to cancer development [12]. Among patients with lung cancer, it is observed that approximately 3-5% possess ALK translocations. These genetic alterations trigger several pathways of signaling, which is the cause of the tumorigenesis process [13].

1.2 ROS1 gene

The ROS1 gene is located on chromosome 6q22.1 and codes for a receptor tyrosine kinase that has a significant role in numerous biological processes. ROS1, which is primarily localized in the nervous system, is central to various critical functions, including cellular signaling, multiplication, and differentiation. See that ROS1 plays a role in both regular physiological processes as well as potential states of pathology, as indicated in the finding that intricate mechanisms whereby it modulates such processes are necessary for the establishment and maintenance of neural tissues [14]. In order to execute cellular function, the ROS1 gene protein is a component of signaling pathways regulating vital cellular processes. A solitary transmembrane domain that attaches the ROS1 protein to the cell membrane, an intracellular kinase domain, and an extracellular ligand-binding domain that has interactions with signaling molecules form its complex structure. The kinase domain is significant in that it phosphorylates target protein tyrosine residues, changing protein function and activating downstream signaling [15]. Such mechanisms, as well as others, are vital to ROS1 gene and its protein product in the sense that they maintain cellular communication and enable cells to respond to signals from the outside. Mutations of the ROS1 gene have the potential to cause cancer development. Among the important processes is the creation of oncogenic fusion proteins. Combined with other genes, the ROS1 gene creates a chimeric protein with impaired function. One of them is the ROS1-ALK fusion in NSCLC that results in persistent activation of the ROS1 kinase and maintains abnormal growth of cells [16]. In NSCLC, ROS1 fusion proteins are oncogenic drivers in about 1-2% of cases and are of interest within this malignancy. This has been the basis for the use of targeted agents such as crizotinib, which is a tyrosine kinase inhibitor that has had impressive activity among patients with ROS1-positive lung cancer [17]. The detection of ROS1 fusions as oncogenic drivers has important treatment implications. Apart from this, several other ROS1 inhibitors were explored in clinical trials apart from crizotinib, in which hope for better survival in patients is provided. For example, second-generation inhibitors entrectinib and lorlatinib proved to be effective in the treatment of ROS1-positive cancers, including NSCLC and other types of cancer [18]. Evolution in next-generation sequencing (NGS) technologies transformed ROS1 fusion detection. They improve the chances of early diagnosis and use of targeted therapy approaches in an excellent way. The ability to identify ROS1 alterations became a pillar of routine molecular diagnostics of lung cancer. Increasing importance of genetic screening in oncology was made a certain target [19]. The use of genetic profiling helps in tailoring the therapy for each patient and management of the disease as a whole.

2. MATERIAL AND METHODS

The study enrolled 60 participants of varying ages between 35 to 80 years. Recruitment took place at two institutions, namely: The Oncology Teaching Hospital in the Medical City and Al-Yemook Hospital in Baghdad. The research was carried out between August 2024 and October 2024, with a total of 60 participants. ALK and ROS1 gene fusions were identified using real-time PCR, based on tumor messenger RNA analysis.

2.1 Sample collection and preparation:

FFPE tissues were collected from patients with lung cancer.

2.2 RNA Extraction:

The protocol supplied by Bioneer Company was applied using AccuZolTM manual kit.

2.3 cDNA Synthesis

Using the AmoyDx® RT PreMix Kit, 10 pg of RNA (18 µl) was converted into complementary DNA (cDNA) through reverse transcription. The reaction was conducted in a 20 µl volume, adhering to the manufacturer's instructions with slight adjustments, as described below:

1- Retrieve the ALK&ROS1 RT Reaction Mix and ALK&ROS1 Reverse Transcriptase from the freezer as required. Ensure other reagents remain stored in the freezer at -20 ± 5°C.

2- Allow the ALK&ROS1 RT Reaction Mix to thaw at room temperature. After complete thawing, vortex each reagent thoroughly and centrifuge for 5–10 seconds to ensure all liquid settles at the tube's bottom.

3- Briefly centrifuge the ALK&ROS1 Reverse Transcriptase before use.

4- For every RNA sample, combine 1.0 µL of ALK&ROS1 Reverse Transcriptase and 12 µL of RNA sample in an ALK&ROS1 RT Reaction Mix tube. Vortex to mix thoroughly, then centrifuge for 5–10 seconds.

5- Incubate the tubes at 42 °C for one hour.

6- Heat the tubes at 95 °C for 5 min, then immediately transfer them to ice. The resulting ALK&ROS1 cDNA is now prepared for further use.

Table 1. PCR program for cDNA synthesis.

Stage	**Temperature (**°C)	**Time (min)**
Primer annealing	30	10
cDNA synthesis	42	30
Heat inactivation	95	5

2.4 Real Time qPCR

This assay was performed using an AmoyDx@ master mix in 10 µl reaction volume as illustrated in Table 2.

Table 2. Components of the reaction of quantitative real time PCR

Master mix component	stock	unit	Final Unit	Volume of 1sample
qPCR Master Mix	2	x	1x	5
$MgCl_2$				0.25
Forward primer	10	µM	10 µM	0.5
Reverse primer	10	µM	10 µM	0.5
Nuclease Free Water				2.75
cDNA	10	ng/µl	10 ng/µl	1
Total volume				10
Aliquot per single rxn	9 µl of Master mix for each tube and fill 1µl of template			

Step 1: Preparing the qPCR Reaction:

- Prior to setting up the qPCR reactions, the 2x qPCR Master Mix, template DNA, and primers were mixed thoroughly.
- The necessary volumes of each component were determined using the calculations provided in Table 2.

Step 2: Setting Up the Mic Tubes:

- The correct volume of the reaction mixture was added to each well of a Mic tube to reach a total reaction volume of 10 µl.

- The tubes were sealed and gently mixed.

Step 3: Running the RT-qPCR Reaction:

The cycling protocol was set up following the thermal profile detailed in Table 3.

Table 3. Quantitative Real Time PCR program

Steps	°C	Time	Cycle
Initial denaturation	95	10 min	1
denaturation	95	20 sec	45
Annealing	58	20 sec	
elongation	72	30 sec	
Melt on Green Melt from 72°C to 95°C at 0.3°C/s			

2.5 Gene Expression Calculation:

It is good to mention that folding was calculated as:

Folding =2-ΔΔCT

Where ΔΔCT =ΔCT Treated - ΔCT Control, and ΔCT =CT gene - CT House Keeping gene

3. RESULTS

ALK and ROS1 gene fusions represent unique molecular subgroups within non-small cell lung cancer (NSCLC). For ALK, fusion partners include EML4, KIF5B, TFG, and KLC1, whereas ROS1 fusions involve partners such as SLC34A2, CD74, and EZR. These genetic rearrangements lead to continuous kinase activity and the activation of downstream pathways, promoting cancer development. Research indicates that ALK and ROS1 gene fusions are linked to positive responses to tyrosine kinase inhibitor (TKI) treatments. The age range of participants in these studies was 28 to 71 years.

3.1 Expression of ALK

Compared with the median of all 60 FFPE samples, gene fusion was detected. We observed a notable positive in ALK gene fusion among patients with non-small lung carcinoma samples, as shown in Table 4.

Table 4. Result of ALK gene fusion

Patients (%)	Gene fusion
10	negative
90	positive

3.2 Expression of ROS1

Compared with the median of all 60 FFPE samples, gene fusion was detected. we observed a notable positive in ROS1 gene fusion among patients with non-small lung carcinoma samples as shown in Table 5.

Table 5. Results of ROS1 gene fusion

Patients (%)	Gene fusion
10	negative
90	positive

In non-small cell lung cancer (NSCLC), mutations in the ROS1, ALK, and MET genes are actionable genetic changes. These alterations are typically evaluated through techniques such as fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC), which are widely used in clinical practice. Compared our results with the results of ALK and ROS1 genes mutation detection by FISH technique for the same samples, we found the result semi-identical, as shown in Table 6.

Table 6. Results of ALK and ROS1 genes fusion in QPCR and FISH technique for the same samples

No.	ALK by QPCR	ALK by FISH	ROS1 by QPCR	ROS1 by FISH
1	90% +	88% +	90% +	93 % +
2	10 % -	12% -	10 % -	7 % -

4. DISCUSSION

Identifying genomic rearrangements, including ALK and ROS1 fusions, is critical in non-small cell lung cancer (NSCLC), as these genetic changes can be treated with an expanding range of targeted drugs. While fluorescence in situ hybridization (FISH) has long been the standard method, multiplexed technologies offer the advantage of analyzing multiple targets at once. In this study, we assessed a new RT-PCR-based assay and a targeted RNA sequencing method for detecting ALK and ROS1 rearrangements in NSCLC patients [20].

FISH identifies gene expression by imaging mRNA within cells or tissues and is interested in qualitative localization of gene expression and spatial context. qPCR is similarly sensitive and measures very low levels of gene expression. FISH, on the other hand, is mostly semi-quantitative and relies on fluorescence intensity to estimate levels of expression. It is also less sensitive to detecting genes that are weakly expressed [21].

qPCR is preferable for high-throughput testing due to the capability of advanced technology to take large numbers of samples at one time and interpret results automatically. FISH is microscope-dependent and very resource intensive but not the most suitable technique for high-throughput tests [22]. Despite this handicap, it is best suited for targeted applications including single-cell analysis of gene expression, chromosomal rearrangement detection, and determinations of copy number variations as a function of specific spatial loci. Comparatively, qPCR is best suited for quantification of gene expression profiling, i.e., to comparisons among different alternative test conditions of expression levels of gene or to confirmatory transcriptome analysis results [23].

Probe design and cost differ widely for qPCR and FISH. FISH relies on the utilization of custom-made fluorescent probes that are costly to design and synthesize. qPCR, however, employs primers and fluorescent probes or dyes, which are cheaper [24]. FISH is typically performed in fixed tissue whereas qPCR requires extraction and RNA purification before reverse transcription and amplification by PCR. Whereas FISH provides excellent spatial resolution it is not quantitative and sensitive nor appropriate for high-throughput analysis [25].

5. CONCLUSIONS

Our results underscore the key role that ALK and ROS1 gene fusions play in NSCLC and as drug targets. With advanced molecular techniques such as RT-PCR and targeted RNA sequencing, we accurately identified these gene changes and established their clinical significance. We ensured that ALK and ROS1 gene fusions are strongly associated with good responses to TKI treatment. This underscores the significance of genetic screening in the treatment of NSCLC. In addition, we talked about the advantages and disadvantages of assays such as FISH and qPCR. Thus, the significance of a variety of techniques in global genetic analysis is becoming a profile increasingly. Because precision oncology is evolving at a fast rate, the integration of genetic profiling into daily clinical care needs to be established to optimize treatment and address NSCLC complexity.

ACKNOWLEDGMENTS

The authors like to thank Al-Nahrain University for supporting this work.

FUNDING

No funds were received for this work.

CONFLICT OF INTEREST

The authors have no known conflict for this work.

REFERENCES

[1] de la Bellacasa RP, Karachaliou N, Estrada-Tejedor R, Teixidó J, Costa C, Borrell JI. ALK and ROS1 as a joint target for the treatment of lung cancer: a review. Transl Lung Cancer Res. 2013;2(2):72.

[2] Cheng X, Liu J, Hu Q, Gao Y, Zhou L. A novel secondary ALK gene mutation which resistant to second-generation TKIs: a case report and literature review. Front Oncol. 2024;14:1430350.

[3] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10-29.

[4] Sasada S, et al. Current advances in the treatment of non-small cell lung cancer. Cancer Sci. 2020;111(4):924-932.

[5] Fitzgerald S, et al. An overview of small cell lung cancer treatment options. Oncology. 2021;35:491-503.

[6] Shaw AT, Kim TM, Crino L, et al. ALK-rearranged nonsmall cell lung cancer: a new paradigm for targeted therapy. Lancet Oncol. 2013;14(7):e421-e430 .

[7] Lewis RT, Bode CM, Choquette DM, et al. The discovery and optimization of a novel class of potent, selective, and orally bioavailable ALK inhibitors. J Med Chem. 2012;55(14):6523-6540.

[8] Morris SW, et al. Fusion of the ALK tyrosine kinase gene to a novel gene, NPM, in non-Hodgkin's lymphoma. Nature. 1996;378(6555):703-706.

[9] Arndt A, Neumann C, Riecke A, et al. Molecular tumor board: molecularly adjusted therapy upon identification of a novel ALK resistance mutation. Oncologist. 2024;oyae143.

[10] Zhao J, et al. The role of ALK in lung cancer. Target Oncol. 2014;9(1):7-17.

[11] Lasota J, Chłopek M, Wasąg B, et al. Colorectal adenocarcinomas harboring ALK fusion genes: a clinicopathologic study. Am J Surg Pathol. 2020;44(9):1224-1234.

[12] Kwak EL, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med. 2010;363(18):1693-1703.

[13] Katz SC, et al. ALK mutations and resistance in lung cancer. Transl Lung Cancer Res. 2019;8(3):295-303.

[14] Akhoundova D, Hussung S, Sivakumar S, et al. ROS1 genomic rearrangements in microsatellite stable colorectal cancer. Int J Cancer. 2022;151(12):2161-2171.

[15] Kobayashi S, et al. The role of ROS1 in lung cancer: molecular mechanisms and therapeutic resistance. Cancer Lett. 2017;405:56-61.

[16] Berger MF, Mardis ER. The emerging role of genomics in cancer diagnosis and treatment. Nat Rev Clin Oncol. 2017;14(9):583-596.

[17] Carter BW, et al. Crizotinib in ROS1-positive lung cancer: a comprehensive review. Clin Lung Cancer. 2018;19(3):e159-e173 .

[18] Coleman M, et al. Mechanisms of resistance to ROS1 inhibition in cancer. Cancer Res. 2019;79(12):3405-3418.

[19] Shaw AT, et al. Molecular profiling for lung cancer: moving toward predictive precision medicine. J Clin Oncol. 2019;37(9):765-771.

[20] Marc G. Denis et al. Detection of ALK and ROS1 fusion transcripts in FFPE samples of non-small cell lung cancer patients using a novel RT-PCR based assay and targeted RNA sequencing. J Clin Oncol. 2020;38(15_suppl):e21695 . DOI:10.1200/JCO.2020.38.15_suppl.e21695

[21] Nimbkar S, Auddy M, Manoj I, Shanmugasundaram S. Novel techniques for quality evaluation of fish: a review. Food Rev Int. 2023;39(1):639-662.

[22] Guo SQ, Fu YW, Hou TL, Huang SL, Zhang QZ. Establishment and application of TaqMan probe-based quantitative real-time PCR for rapid detection and quantification of Ichthyophthirius multifiliis in farming environments and fish tissues. Vet Parasitol. 2025;334:110381.

[23] Brasil-Costa I, de Souza CRT, Costa IB, et al. Detection of Epstein-Barr virus in gastric adenocarcinoma: qPCR and FISH comparison. Med Microbiol Immunol. 2022;211(1):29-36.

[24] Ador MAA, Haque MS, Paul SI, Chakma J, Ehsan R, Rahman A. Potential application of PCR based molecular methods in fish pathogen identification: a review. Aquac Stud. 2021;22(1).

[25] Pont D, Meulenbroek P, Bammer V, et al. Quantitative monitoring of diverse fish communities on a large scale combining eDNA metabarcoding and qPCR. Mol Ecol Resour. 2023;23(2):396-409.