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Research Article

Gene Expression of the MLH1 gene is regulated by non-coding RNA in patients with lung cancer

Mohammed S. Khudher[1] and Owayes M. Hamed[1]

[1]College of Science, Biology Department, University of Mosul, 4002, Mosul, Iraq

mohammed.23scp30@student.uomosul.edu.iq

owsbio31@uomosul.edu.iq

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Abstract- Lung cancer is a common cancer worldwide and can occur due to various causes, one of which is the MMR mismatch repair system. This system repairs mismatched nucleotides in the DNA strand during replication. MLH1, a key gene in the MMR system, is essential in repairing mutations in lung cancer patients. Certain non-coding RNA genes, such as snoRNA and FOXD2-AS1, can regulate the expression of this gene. These non-coding RNAs influence MLH1 gene expression in lung cancer patients. The examination targeted at detecting the impact of miRNA and non-coding RNA on MLH1 gene expression rates in lung cancer patients situated within Dohuk City. The study included 70 patients aged between 45 and 65 from September to October 2024, referred to AZADI Hospital. The sample was divided into two groups: one included 55 lung cancer patients, another group included 15 individuals and was considered the control group. A total of 5 ml of blood was drawn from each patient and segmented into two parts. The first part was put in EDTA for DNA extraction, while the second part was placed in a Trizol tube for mRNA and non-coding RNA extraction. The study consisted of two stages: first, detecting the level of MLH1 gene expression; second, assessing the level of non-coding RNA influencing gene activity. The results showed that the MLH1 gene expression value was 0.38, while the gene expression values for non-coding RNAs were 4.4 for snoRNA and 4.2 for FOXD2-AS1.

Keywords: Lung Cancer, Gene Expression, Non-Coding RNA Genes, MLH1 Gene, MMR System, Snorna-21 And FOXD2-AS1, Qrt-PCR.

I. INTRODUCTION

Worldwide, lung cancer produces the most cancer-related deaths because non-small cell lung cancer (NSCLC) appears in 80-85% of cases and small cell lung cancer (SCLC) emerges in 13-15% of patients (1).

The leading risk factor for developing SCLC and squamous cell carcinoma arises from tobacco smoking, along with radon exposure and occupational hazards, and air pollution as other causes. (2)
The fundamental elements of cancer metastasis and its evolutionary process include biological heterogeneity, as well as the genetic landscape. Intratumor heterogeneity develops due to genetic, epigenetic, and environmental factors. (3)
Lung cancer is one of the most common genetic disorders, and the molecular pathogenesis of lung cancer is quite complicated and heterogeneous. Lung cancer can develop as a result of several genetic and epigenetic changes (e.g., point mutations, amplifications, insertions, deletions, and translocations); it is particularly associated with the activation of pathways that promote growth and the inhibition of tumor suppressor pathways. (4) Studies indicate that adenocarcinomas and squamous cell carcinomas, both subtypes of NSCLC, are quite different regarding their molecular features. The precursor mutations and their prevalence in NSCLC by histologic subtype. Smoking is the most important risk factor for lung cancer worldwide; 85% of lung cancers are caused by the carcinogens found in tobacco smoke. The differences in smoking habits are found to be associated with the molecular differences. (5).
Cancer formation results from the abnormal process that disturbs cell cycle operations. The formation of lung cancer begins with mutations in the MYC, BCL2, and p53 genes in small cell lung cancer (SCLC), while non-small cell lung cancer (NSCLC) originates from mutations in the EGFR, KRAS, and p16 genes. (6.7) DNA MMR deficiencies change how sequences of DNA repeats appear within tumor cells. The DNA replication process stimulates DNA replication errors to begin forming before they multiply within the process. Carcinogenesis occurs when genetic coding areas of oncogenes and tumor suppressor genes develop mutations due to this mechanism.
Modern researchers have established DNA MMR deficiency as an essential factor in determining the success of immunotherapy treatment. The purpose of this study is to describe how often DNA MMR deficiency exists during post-surgical lung carcinoma LC analysis and explore possible links between this and clinical pathology features. (8) The MLH1 gene functions to specify the creation of a protein crucial for DNA repair operations. The DNA MMR protein functions to fix mistakes that occur in DNA duplication, which precedes cellular division. The MLH1 protein bonds with the PMS2 protein (originating from the PMS2 gene) to produce the dimer two-protein configuration.This complex functions as the coordinator for proteins that fix mistakes that occur during the process of DNA replication. The correction process requires removing an erroneous DNA section, followed by integrating the corrected DNA sequence into the same location. Among the mismatch repair (MMR) genes, the MLH1 gene is an essential component. (9)
The nuclear noncoding RNA class known as SnoRNAs exists in intronic regions of specific lengths between 60 to 300 nucleotides. The splicing process, together with exonucleolytic processing, transforms snoRNA into functioning mature small nucleolar ribonucleoproteins (snoRNPs). Box C/D snoRNAs and Box H/ACA snoRNAs represent the major families of snoRNAs, which perform 2′-O-methylation and pseudouridylation functions, respectively. Each family type contains different conserved sequences, which include Box C and Box D motifs at the ends for C/D snoRNAs, but two hairpin structures composed of Box H and Box ACA motifs in H/ACA snoRNAs (Ganot, Caizergues-Ferrer, & Kiss, 1997). Research from the beginning period successfully identified the primary nucleolar function of snoRNAs because investigators discovered them in this cellular region while they acted on post-transcriptional ribosomal RNA (rRNA) sequences. (10)
FOXD2-AS1 is a long non-coding RNA located on chromosome 1p33 that functions as an oncogene in various cancers (11). It is upregulated in multiple malignancies, including colorectal, hepatocellular, breast, lung, glioma, and thyroid cancers. (12.13.14.15.16). FOXD2-AS1 promotes cancer progression by enhancing cell proliferation, migration, invasion, and stemness while inhibiting apoptosis. (17)

Genetic mutations cause lung cancer in addition to alterations in the MLH1 gene that belongs to the mismatch repair system. The research adopted two main targets to evaluate repair gene expression levels alongside studying regulatory processes and non-coding RNA types. The research helps explain how DNA mutations could disrupt particular gene regulatory functions, thereby causing specific functional loss of the

gene. The research contains a summary followed by an introduction and targets articulated within. The following section will discuss materials and will analyze methods, followed by results, and create a discussion.

II. MATERIALS AND METHODS

2.1 Purpose of Study

Seventy (70) patients between the ages of 45 and 65 were examined between September and October of 2024 and were referred to the AZADI specialized hospital based on private pathological investigation facilities in Duhok. The sample was divided into two categories. The first contained 55 patients with lung cancer, while the second contained 15samples considered as control.

2.2 Blood Sample Collection

Fifteen milliliters of venous blood taken from male patients were distributed into a two-segmented collection system. An EDTA tube received the first sample due for DNA extraction, and the second sample was placed in trizol tube for RNA extraction.

2.3 RNA Extraction

The collected blood samples were combined with trizol, which included 750 ml of reagent and 250 ml solution for both patient and control blood specimens. The extracted RNA purity measurement relied on the Nanodrop device following the RNA extraction process using kits provided by Transgenbiotech. Research on the MLH1 gene expression levels used q-PCR analysis. The q-PCR reactions required specific housekeeping genes combined with targeted gene primers, which followed the patterns shown in Table 1.

Table1: the primers of MLH1 used in RT-PCR reaction [25](18)

Primer	Sequence
GAPDH Forward	5′-GCGAGATCGCACTCATCATCT-3′
GAPDH Reverse	5′-TCAGTGGTGGACCTGACC-3′
Mlh1 RT-qPCR forward	5′-TCCCGAAAGGAAATGACT-3′
Mlh1 RT-qPCR reverse	5′-TTGGTGGTGTTGAGAAGGT-3′

The table show primers of housekeeping gene (GAPDH) and mismatch repair gene (Mlh1) use to measure gene expression.

Real-time PCR is a specific method for detecting gene expression and non-coding RNA levels. Table 2 presents the primers for non-coding RNA (snoRNA-21, FOXD2-AS1) used in the RT-PCR reaction.

Table2: The primers of non-coding RNA used in the RT-PCR reaction [25](18)

Primer	Sequence
U6-forward H.K gene	5´ GTGCTGCTTGGGCAGCA 3´
U6-reverse H.K gene	5´ GAAATATGGAACGGTTC 3
snoRNA-21-forward	5´GTTTGGTAGCTTATGAGACTGA´3
snoRNA-21-reverse	5´GTGCAGGGTCGGAGGT´3
FOXD2-AS1 lncRNA forward	5′-TGGACCTAGCTGCAGCTCCA-3′
FOXD2-AS1 lncRNA reverse	5′-AGTTGAAGGTGCACACACTG-3′

The table shows primers of non-coding RNA genes regulate the mlh1 gene include:-housekeeping gene(U6) and snoRNA-21 which should be designed by us, and lncRNA (FOXD2-AS1) it is taken from research. (18)

The final reaction volume was 20µl, as shown in the following table:

Table 3: The final reaction volume

Component	Volume
SYBR qPCR Mix	10µl
RT-FP	0.5µl
RT-RP	0.5µl
cDNA Template	4µl
Nuclease-Free Water	5µl

In this table show reaction volume include SYBR qPCR Mix that is master mix with SyberGreen is fluorescent dye and also Forward primer and Reverse primer of gene and cDNA templet use to annealing of primer and amplification also use distilled water.

SYBR Green-based qPCR Master Mix → SYBR qPCR Mix

Forward Primer for Reverse Transcription → RT-FP

Reverse Primer for Reverse Transcription → RT-RP

Complementary DNA (cDNA) Template → cDNA Template

Molecular Biology-Grade Water / Nuclease-Free Water → NF Water or D.W(distilled water)

Table 4: presents the program used in the q-PCR reaction.

Stage	**Temperature**	**Time**
Pre denaturation	95 °C	10 min
Denaturation	95 °C	15 sec
Annealing /Extension	60 °C	1 min
Melting curve analysis	95 °C 55 °C 95 °C 55 °C	15 sec 1 min 15 sec 15 sec

The qPCR program progression appears in the table.
The first step of pre-denaturation heats up DNA polymerase and causes DNA strand unwinding in denaturation, then during annealing the DNA interacts with its primer before extension creates a new DNA strand leading to melting curve analysis tracking DNA denaturation through temperature-based strand separation.

2.4 Determine the expression rate of genes through the calculation of target gene CT values versus housekeeping CT values

The mathematical formula calculates gene expression rates from target gene CT values within reference gene CT ranges of patient versus control groups.

1	RQ	2*-△△CT
2	△△CT	△CT (Test sample)- △CT(control sample)
3	△CT(test sample)	CT(target gene in test)-△CT(reference gene in test, H.K gene)
4	△CT(control sample)	CT(target gene in control)-△CT(reference gene in control, H.K gene)

RQ = 1-2 normal RQ > 2 overexpression RQ < 1 low expression

III. RESULTS AND DISCUSSION

3.1 Measuring the gene expression level of the MLH1

The obtained study results led to detect different expression levels for the MLH1 gene among the patient and control groups. The CT values and gene expression levels of MLH1, together with housekeeping gene expression data between lung cancer patients and the control group appear in Table 5. Figure 1 illustrates the activity levels of MLH1 gene.

Table 5: Measuring the gene expression level of the MLH1

Sample	CT target	CT housekeeping	ΔCT target	ΔCT control	ΔΔCT	Gene expression
Control	35.2	30.75	4.45	4.45	0	1
Patients	36.9	31.06	5.84	4.45	1.39	0.38

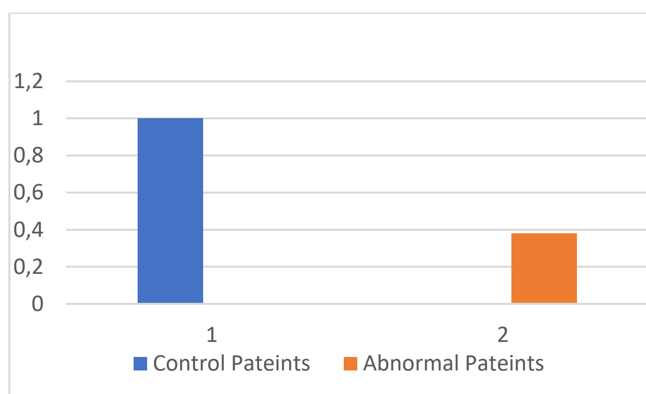

Figure 1. The level of MLH1 gene expression

Figure 1: shows the level of MLH1 gene expression in lung cancer patients and the control group. The study outcome demonstrated decreased MLH1 gene expression levels at 0.38 whereas control group participants showed 1 level of gene expression considering their absence of health issues. Pathological condition development has this factor as one of its primary causes.

This study showed the MLH1 gene expression of participants to be at 0.38. The control group served as the reference for pathological condition development through usage of patients without health issues whose baseline value was set to 1. The low levels of MLH1 gene expression disable regular cellular functions and cancer suppression since mutations occur because genomic abnormalities control this gene through non-coding RNA sequences (snoRNA-21, FOXD2-AS1). The hMLH1 promoter hypermethylation activates gene expression reduction thus disabling mismatch repair functions and enabling cancer development primarily in colorectal cancer cases, as reported in the study. (19)

3.2 Result of the level of gene expression for snoRNA-2

Research data indicates that Table 7, combined with Figure 3 presents gene expression of snoRNA-21 in lung cancer patients and control participants.

Table 7: Result of the level of gene expression for snoRNA-2

Sample	CT target gene	CT housekeeping gene	Δ CT target gene	Δ CT control	Δ Δ CT	Gene Expression folding
Control	24.3	31.9	-7.6	-7.6	0	1
Patients	22.52	32.28	-9.76	-7.6	-2.16	4.4

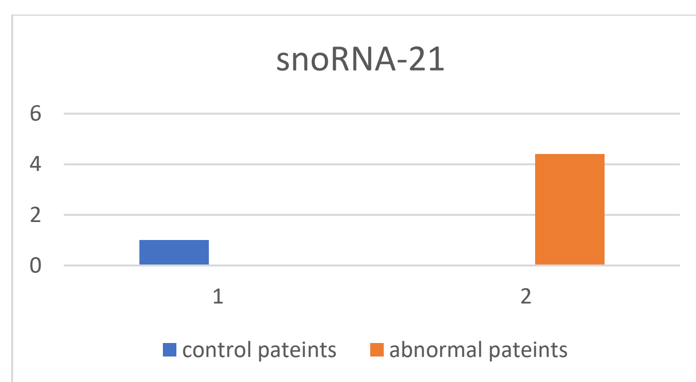

Figure 3: The level of gene expression for snoRNA-21 and control group

The research data showed that lung cancer patients displayed snoRNA-21 gene expression levels at 4.4 while the control group maintained levels at 1.0. The interaction of snoRNA-21 with MLH1 mRNA triggers post-transcriptional repression while simultaneously reducing protein production and promoter methylation from snoRNA-21 helps generate MLH1 promoter hypermethylation. which silences the gene and prevents its transcription, in normal physiological processes, snoRNAs stably exist by forming snoRNPs through interactions with specific proteins, and they subsequently participate in 2'-O-methylation or pseudo uridylation of rRNA (Henras et al., 2004). (20)

3.3 Result of the level of gene expression for FOXD2(lncRNA) gene

The analysis of FOXD2(lncRNA) gene expression levels appeared in Table 8 along with Figure 4 between lung cancer patients and controls.

Table 8: Result of the level of gene expression for FOXD2(lncRNA) gene

Sample	CT target gene	CT housekeeping gene	Δ CT target gene	Δ CT control	Δ Δ CT	Gene Expression folding
Control	30.8	28.9	1.9	1.9	0	1
Patients	32.2	32.36	-0.16	1.9	-2.06	4.2

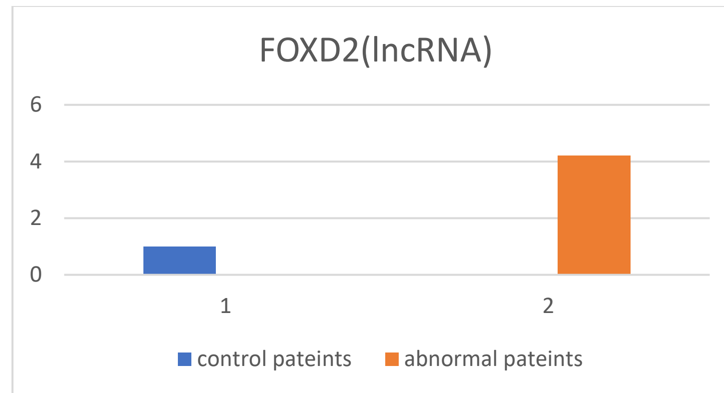

Figure 4: The level of gene expression for FOXD2(lncRNA)and control group

Research findings demonstrated significantly higher FOXD2(lncRNA) gene expression levels in lung cancer patients, amounting to 4.2, compared to control participants with expression levels at 1.0. FDX2-AS1 enhances MLH1 silencing through DNA promoter methylation, which stops gene expression and impedes MLH1-regulating miRNAs. This leads to lower MLH1 levels, genomic instability, and improved cancer cell survival.

The DNMT1 enzyme was drawn to the MLH1 promoter region through the action of the lncRNA FOXD2-AS1, which then suppressed transcription activities and genetic material production. Past research confirmed how DNA methylation regulates multiple cellular processes, including the cell cycle, cell invasion, and human cancer metastasis. (21)
This study indicates that this gene is responsible for lung cancer. The study included only male patients suffering from this cancer in Duhok Governorate.

IV. CONCLUSION

The study provides vital insights regarding non-coding RNA effects on MLH1 gene expression in lung cancer patients in Dohuk City by evaluating and silencing RNAs (snoRNAs) and FOXD2-AS1 regulatory mechanisms. The research data demonstrated significant MLH1 gene expression modification that corresponded to regulatory non-coding RNA value adjustments involving snoRNA and FOXD2-AS1. Research indicates that regulatory non-coding RNAs function as vital control elements for mismatch repair activities that might contribute to lung cancer occurrence and disease evolution by managing MLH1 gene expression. Research findings establish that studying molecular mechanisms of cancer initiation requires investigating mismatch repair DNA deficiency regulation by RNA regulatory molecules. Scientific teams exploring diagnostic biomarkers and lung cancer therapy targets have access to this research data to establish proposed methods using non-coding RNA systems to modulate MLH1 expression levels. Additional research involving extensive study samples from various patient demographics is needed to assess non-coding RNA therapeutics for lung cancer treatment.

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