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# The Role of miR-142 and miR-195 in Regulating Apoptotic Pathways in Non-Small Cell Lung Cancer

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## Abstract

Lung cancer remains the leading cause of cancer-related mortality worldwide. non-small cell lung cancer (NSCLC) accounts for the majority of cases, despite advancements in treatment modalities, including chemotherapy, targeted therapy, and immunotherapy. The prognosis for NSCLC patients remains poor, largely due to challenges such as drug resistance and relapse. This study investigates the role of microRNAs (miRNAs) in the apoptotic pathways associated with NSCLC, focusing specifically on miR-142 and miR-195. These miRNAs have been implicated in regulating critical cellular processes, including proliferation and apoptosis.

This study employed 5-fluorouracil (5-FU), a common chemotherapeutic agent, to assess its cytotoxic effects on the A549 NSCLC cell line. The half-maximal inhibitory concentration (IC50) for 5-FU was determined to be 10.84 µg/mL after 48 hours. Morphological studies revealed significant changes in cell structure post-treatment, while flow cytometry indicated that 31.3% of cells underwent early apoptosis. Quantitative real-time PCR (qRT-PCR) analyses demonstrated a significant upregulation of p53 and Bax, alongside a downregulation of Bcl-2 in treated cells, suggesting a shift towards pro-apoptotic signaling. Notably, miR-142 expression was significantly increased, while miR-195 showed a non-significant upward trend.

These findings underscore the potential of miRNAs as therapeutic targets in NSCLC, particularly in overcoming drug resistance by modulating apoptotic pathways. Further exploration of miRNA-based therapies may yield innovative strategies to enhance treatment efficacy and improve patient outcomes in lung cancer.

## 1. Introduction

Lung cancer is the primary cause of mortality in the United States and around the globe(1), with an incidence rate of 14.5 men and 8.4 women per 100,000 individuals. It can be classified into two main groups based on pathologic features: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC accounts for 80-85% of all lung cancer cases (2). The most common cause is long-term exposure to tobacco carcinogens and environmental pollution, genetic susceptibility, chronic obstructive pneumonia, and infection (3).

Diagnostic procedures include sputum cytology, tissue biopsy, and imaging tests such as bronchoscopy, X-ray, MRI, positron emission tomography, or computed tomography scan (4). Treatment strategies include surgical resection, chemotherapy, targeted therapy, and radiotherapy. However, despite these options, the prognosis remains distressingly poor with a low 5-year survival rate (5).

Various challenges such as drug resistance after chemotherapy, relapse and cell resistance after radiotherapy, genetic mutations causing resistance in targeted treatments, and also safety side effects, have created many challenges to lung cancer treatment (6).Therefore, it is necessary to discover more effective, safer, and more affordable therapeutic agents (7).

At present, accumulating evidence has shown that non-coding small RNAs play an important role in NSCLC pathogenesis, which provides new insights into the treatment of this disease (8). miRNA-based therapies show potential A lot has been done to develop new treatments for NSCLC (9).

MiRNAs belong to the large family of non-coding RNAs of 20–24 nucleotides that regulate gene expression at the posttranscriptional level by binding to the 3′-UTR of target mRNAs (10). and play a role in modulating several key mechanisms in cells, such as pharmaceutical resistance, proliferation, differentiation, inflammation, and apoptosis (11). The eukaryotic genomes contain transcriptional units that will generate miRNAs. These information units, which appear isolated or clustered, can be located in several genomic territories, including intergenic regions, coding, and non-coding genes. After transcription, the typical hairpin-loop secondary structure present in primary miRNAs (pri-miRNAs) is recognized and excised by the microprocessor complex (composed of DGCR8 and Drosha). The generated precursor miRNAs (pre-miRNAs) will be exported to the cytoplasm and further processed by the Dicer nuclease to generate a dsRNA. The mature miRNA chain will be selected by Ago2 and engaged together into the RNA-induced silencing complex (RISC) to exert its regulatory action (12).

Several studies confirm that microRNAs play an important role in the initiation and progression of cancer, and many of them act as oncogenes and tumor suppressors. On the other hand, microRNAs are important stimulating factors that can act as biomarkers in the diagnosis and prognosis of various types of cancer, and in many cases, the occurrence of mutations in microRNAs and open-reading templates can lead to cancer (13).

In recent years, several miRNAs have been reported to be dysregulated in various cancers. Most recent findings have shown that miR-142 gene, located at chromosome 17q22, is involved in cellular migration, proliferation, and apoptosis in different human cancers (14). Consistently, Xiao et al demonstrated that miR-142-3p was lowly expressed in NSCLC tissues and cells, and ectopic expression of miR-142-3p induced NSCLC cell proliferation reduction and apoptosis increase. These reports indicate that miR-142 plays an important role in NSCLC progression (15).

Among microRNAs, miR-195 has been reported to significantly impact oncogenicity in various neoplasms by binding to critical genes and signaling pathways, enhancing or inhibiting the progression of cancers. (10)MicroRNA-195-5p (miR-195-5p) has been reported to be a cancer regulator in multiple cancers such as colon cancer, gastric cancer, and so on, yet the function of miR-195-5p and the underlying mechanism in regulating lung cancer still remains to be investigated (16).

some miRNAs (e.g., miR-21 and miR-192), are involved in different cell death pathways. Because the balance between cell proliferation and cell death is pivotal to the homeostasis of the human body, miRNAs that regulate cell death pathways have drawn much attention from researchers. Both apoptosis and microRNAs are important in cancer (17).

Apoptosis is a well-organized and coordinated cellular process that happens in several physiological situations. Aberrant regulation of apoptosis has also been documented in numerous pathological situations, particularly cancer (18). While the extrinsic pathway is stimulated by death receptors, namely Fas, TNF receptors, and TRAILs, the intrinsic pathway is initiated by DNA damage, energy starvation, and hypoxia, which can dephosphorylate and cleave pro-apoptotic proteins, resulting in their recruitment in the mitochondria. Both pro-apoptotic and anti-apoptotic members of the Bcl-2 family proteins regulate intrinsic apoptotic pathways (19).

Bax is a pro-apoptotic protein that plays a pivotal role in controlling apoptosis. It is generally present in the cytoplasm, which is heterodimerized with anti-apoptotic proteins. A low expression of Bax has been drastically linked with NSCLC patients and poor prognosis in NSCLC patients (20).

Lymphoma cell Bcl -2(2B) is an anti-apoptotic promoter encoded by the Bcl -2gene in the human genome and is known as an oncogene.

Bcl2 causes cancer growth and resistance to chemotherapy drugs xenograft models of NSCLC. Bcl2 is a good prognostic marker in NSCLC and is considered a constructive prognostic biomarker in LUSC. express more than The limitation of Bcl-2 and the suppression of Bax expression leads to the homeostatic imbalance of cells and ultimately causes cancer (21).

Studies have shown that mutations in the BCL-2 and BAX proteins are frequently detected in several types of cancers, suggesting that they play crucial roles in elucidating molecular mechanisms driving oncogenic transformation and drug resistance (22).

The p53 gene was the first tumor suppressor gene to be identified, and the discovery that p53 has a role in human cancers has greatly facilitated the research of p53 functions in tumors. p53 plays an important regulatory role in these processes p53 is more like a brake in the cell cycle. The mutation in p53 is acknowledged as one of the most common concurrent genomic alterations in non-small-cell lung cancer (NSCLC)(23).

Caspases, a group of protease enzymes (cysteine proteases), exist as inactive zymogens in the cells and execute apoptosis (programmed cell death). Caspase-3, an executioner caspase, plays an imperative role in apoptosis and becomes a primary target for cancer treatment (24). Tumor necrosis factor (TNF)-alpha is one of the most important cytokines in the immune system (25).Tumor necrosis factor receptors are widely expressed in NSCLC non-small cell lung cancer they become TNF have a consistent role in inflammation and can cause cell death in cancer cells (26).

5-Fluorouracil (5-FU) has been an important anticancer drug to date. With an increase in the knowledge of its mechanism of action, various treatment modalities have been developed over the past few decades to increase its anti-cancer activity. But drug resistance has greatly affected the clinical use of 5-FU. Overcoming this chemoresistance is a challenge due to the presence of cancer stem cells like cells, cancer recurrence, metastasis, and angiogenesis (27).

The expression level of miRNA-142 and miRNA-195 in lung cancer cells treated with 5-FU can have a deep look. It provides the mechanisms of drug resistance and apoptosis in these cells. For example, if it is clear That these miRNAs are downregulated in 5-FU resistant cells, this may indicate that They play an important role in promoting apoptosis and their downregulation in these cells allows them to escape from apoptosis benefits and become resistant to the drug (28).

in this study, we evaluated the expression levels of miRNA-142 and miRNA-195 in lung cancer cells treated with 5-FU, which can provide valuable insights into the role of these miRNAs in apoptosis and drug resistance to give this study can potentially help to formulate new treatment strategies and overcome the problem of drug resistance is effective in lung cancer.

## 2 Materials and Methods

### 2.1 Cell Culture

In this study, cell line A549 was obtained from the central bank of genetic resources and cultured according to the relevant protocols. cell in the environment at a temperature of 37 degrees centigrade, culture with 5% 2CO and 1% amount of penicillin and streptomycin and FBS with 10% DMEM(5 ml of FBS with 500 µl of antibiotics (ml/U 100 penicillin and 100 µg/ml of streptoma) 50 ml of sterile lysine is poured into the falcon tube and then its volume is increased by adding DMEM medium We bring it to 50 ml.) We bring it to 50 ml.)

### 2.2 Estimation Of The Number Of Viable Cells

To estimate the number of living cells during the passage after pipetting the suspension inside the flask was poured into the sterile falcon and for time10 minutes at 1500 rpm We centrifuge. After draining the supernatant solution and adding a complete medium to dissolve the sediment10 microliters of trypsin cells Not with10 microliters of trypan blue dye (0.25%) (mixed and 10 microliters of cell suspension by Elm Neobar were examined by light microscope. The following formula is used to calculate the number of cells in the neobar element)4-: Total number Numbered cells (/)4 counted squares × 10 × (the first volume is ×dilution factor = total number of cells)

### 2.3 MTT ASSAY

After 24, 48, and 72 hours of incubation, the cells were treated with drugs at concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 micrograms/ml were treated in 96 house plates. cells in 37 °C and 5% 2Co and 95% 2O were cultivated. Finally, determine the percentage of cell survival was calculated.

### 2.4 Flow Cytometry (Determining cell apoptosis using PI/FITC-V-Annexin staining technique)

Cells were seeded in six-well culture plates and were treated in complete medium for cell cycle analysis. After 24 h, cells were trypsinized and suspended in 70% ethanol overnight. The cells were stained with Annexin-v and PI and data acquisition was performed in the flow cytometer. In order to quantify the abundance of apoptotic cells, V-Annexin conjugated to fluorescein dye FITC isothiocyanate is used to separate primary apoptotic cells from necrotic cells from propidium. PI iodide is used.

### 2.5 Analysis Of The Expression Level Of Apoptosis Genes

#### 2.5.1 Extraction of Total RNAs

RNA extraction is the first step in examining gene expression. After treatment of cancerous and healthy cells with IC50 concentration of the drug synthesized in the 6 house plate and incubation time, RNA extraction using The Trizol method was performed according to the protocol from the extraction kit (Kimia Andishe Teb)

#### 2.5.2 Assessment of Extracted RNA Quality Using Agarose Gel Electrophoresis

First To perform electrophoresis, it is necessary to prepare agarose gel. 0.25 grams of agarose powder was poured into 25 cc of X1 TBE buffer and dissolved in a microwave until a clear solution was obtained. The amount of 5 microliters of each sample was added to the wells and before pouring it into the wells, it should be mixed with 1 microliter of blue dye be combined and The 28S and 18S RNA bands are indicated.

#### 2.5.3 Assessment of Extracted RNA Quality using Nanodrop

To obtain the concentration and purity of the samples quantitatively at 260 and 280 nm wavelengths nanodrop device was used. Then the optical absorbance (OD) of the sample at the wavelength was measured and the number was 260/280, which indicates the purity of the extracted RNA was observed.

### 2.6 CDNA Synthesis

cDNA synthesis using the Revert cDNA synthesis kit AidTM (Pars Toos Company Tehran-Iran) Contains all the necessary components for converting total RNA into single-stranded cDNA. Because DNA polymerase is not able to use RNA as a template; Another step to PCR has been added. Thus, to prepare cDNA for Real-Time PCR Reveres enzymeTranscriptase inhibitor converts RNA to DNA. In this experiment, to synthesize cDNA, With the help of random hexamer reverse transcription.

### 2.7 Primer Design

For the synthesis of cDNA from (miRNA (mIn this method, specific primers called loop-stem primers are used. These primers are in the form of oligonucleotides with a length of about 50 base pairs of DNA, which are six of their 3' end nucleotides are complementary to the six 3' end nucleotides of the desired miRNA sequence are designed These primers cause binding and identification due to the hairpin structure specific miRNAs.For others, we used the Primer3Plus website and entered our cDNA sequence and we specified primer parameters such as length (typically 18-27 nucleotides), melting temperature, and GC(40-60%)and used NCBI primer blast to confirm design primers(Table 1). desired miRNA sequence are designed These primers cause binding and identification due to the hairpin structure specific miRNAs.For others, we used the Primer3Plus website and entered our cDNA sequence and we specified primer parameters such as length (typically 18-27 nucleotides), melting temperature, and GC(40-60%)and used NCBI primer blast to confirm design primers(Table 1).

### 2.8 Real-time PCR

The Syber Green method will be used to Analysis of the expression level of apoptosis genes. SYBR Green dye binds the minor groove of double-stranded DNA. When Syber Green dye binds to double-stranded DNA,

the intensity of the fluorescence increases. As more double-stranded amplicons are produced, SYBR Green dye fluorescence increases. The GAPDH gene is used as an internal control. Bioneer system96 The Exicycler was optimized as follows: Initial denaturation of template DNA at 95 °C It was done for 10 minutes and we did the second step alternately (40 cycles at 95 degrees Celsius 34 for 20 s, 65 °C for 20 s and 72 °C for 20 s). We calculated using the ΔΔCt method.

## 3. Result

### 3.1 The results of the 5-fluorouracil cytotoxicity method on the A549 cell line using the MTT method

A549 cell line was treated with different concentrations of 0, 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml of the drug, and the effect of this drug on cell growth was determined in three time periods of 24, 48, and 72 hours. For the reasons shown in 1-4, 5-FU (0-10 µg/L) increases the dose and time in A549 cells. The IC50 for 48 hours of 5-FU treatment in A549 cells was 10.84 µg/L. Based on the results, 10 µg/ml 5-FU was used for 48 hours in A549 cells for further experiments (Figure 1).

**Table 1**: Primers list designed for molecular study

| Primer Name | | Sequence (5'to3') | Length | Tm° C | Amplicon size |
|---|---|---|---|---|---|
| GAPDH | Forwad | TGCCTCCTGCACCACCAAC | 19 | 62.79 | 178bp |
| | Reverse | CGGAGGGGCCATCCACAG | 18 | 62.18 | |
| TNF-a | Forwad | CTCTTCTGCCTGCTGCACTTTG | 22 | 62.27 | 135 bp |
| | Reverse | ATGGGCTACAGGCTTGTCACTC | 22 | 62.33 | |
| P53 | Forwad | CCTCAGCATCTTATCCGAGTGG | 22 | 61.05 | 181 bp |
| | Reverse | TGGATGGTGGTACAGTCAGAGC | 22 | 60.9 | |
| Bcl-2 | Forwad | ATCGCCCTGTGGATGACTGAGT | 22 | 63.47 | 127 |
| | Reverse | GCCAGGAGAAATCAAACAGAGGC | 23 | 61.98 | |
| Bax | Forwad | CCACCCTGGTCTTGGATCCAGCCC | 24 | 66.67 | 127 bp |
| | Reverse | CCTGTGCACCAAGGTGCCGGAACT | 24 | 62.50 | |
| CASP3 | Forwad | GGAAGCGAATCAATGGACTCTGG | 23 | 61.54 | 146 bp |
| | Reverse | GCATCGACATCTGTACCAGACC | 22 | 60.80 | |
| miRNA195 | Forward | GGGGTAGCAGCACAGAAAT | 19 | 57.42 | 146bp [73] |
| | Reverse | TCCAGTGCGTGTCGTGGA | 18 | 61 | |
| miRNA142 | Forward | GTCGTATCCAGTGCAGGG | 18 | 62.12 | 134bp [74] |
| | Reverse | GGACGTGTAGTGTTTCCTA | 19 | 64.24 | |
| U6 | Forward | TGCGGGTGCTCGCTTCGCAGC | 21 | 61.5 | 135bp [73] |
| | Reverse | CCAGTGCAGGGTCCGAGGT | 19 | 63.32 | |

### 3.2 Optimizing the appropriate drug concentration on a healthy cell line with the MTT method

Healthy MRC5 cell lines were treated with concentrations of 0, 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml of 5-FU, and the effect of this drug on cell growth was investigated in three periods of 24, 48, and 72 hours. Then, the absorbance was measured at a wavelength of 570 nm. According to Figure 2-4, the results showing IC50 for 24, 48, and 72 hours of 5-FU treatment in MRC5 cells were 66.21, 46.55 and 31.86 µg/ml (Figure 2).

### 3.3 Morphology of A549 cells after treatment with 5-fluorouracil

Morphology of the cells using an inverted light microscope was done to investigate the effect of 5-FU treatment at IC50 concentration on the A549 cell line after 48 hours. It was noted that after 48 hours, the control cells remained small and round, and continued to multiply, but after treatment with 5-FU, the cells were larger, elongated, and less dense compared to the control cells. (Figure 3).

### 3.4 The results of the flow cytometry technique using Annexin V-FITC kit to investigate the induction of apoptosis in cells treated with 5-fluorouracil

The flow cytometry results in Figure 3-4(A) show that 97.7% of the cells survive, which is what we expect from the control cells, most of the cells do not undergo apoptosis, because they were not treated with drugs. Therefore, the number of cells that underwent early and delayed apoptosis were 2.11%, 0.010%, and 0.193% of the cells that underwent necrosis under the influence of the collection and preparation stages, respectively, and this number compared to healthy cells and Live is considered a small amount. The results of apoptosis induction in cells treated with the IC50 dose are presented in Figure 4-3(B).

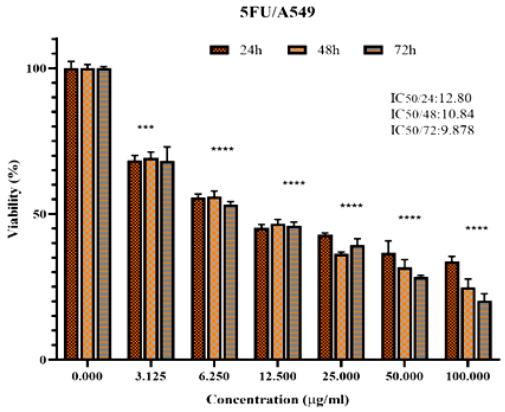


**Figure 1.** The results of the survival percentage of A549 cells after treatment with different drug concentrations for 24, 48 and 72 hours using the MTT method. *P>0.05, **P<0.01, ***P<0.001, ****P<0.0001 are considered significant.

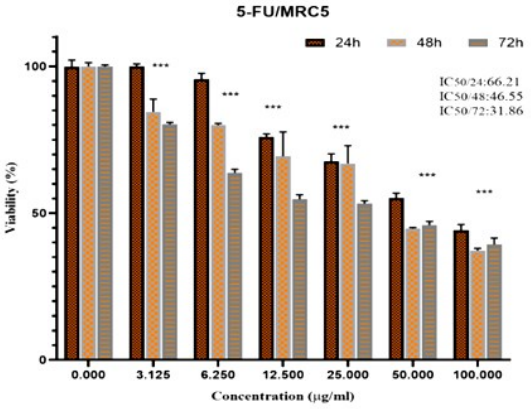


**Figure 2.** The results of the survival percentage of MRC-5 cells after treatment with different drug concentrations for 24, 48, and 72 hours using the MTT method. *P>0.05, **P<0.01, ***P<0.001, ****P<0.0001 are considered significant.

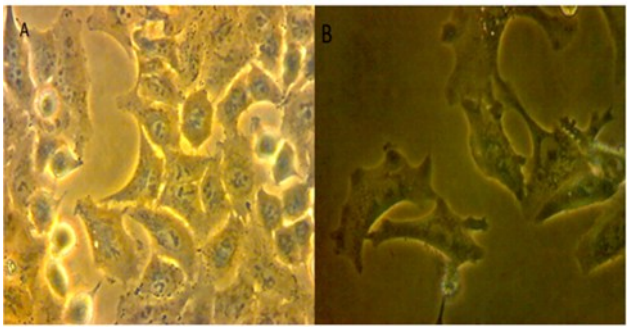


**Figure 3.** A549 cell morphology. A: Untreated cells. B: 5-FU-treated cells at 50 magnification

These results related to calculating the percentage of apoptotic cells by flow cytometry are displayed in the form of the average percentage of cells + standard deviation. According to the results of flow cytometry for A549 cells treated with the drug, the percentage of live cells (region Q4) was 57.9%. The cells that had early apoptosis (Q3 area) were 31.3% and the cells that were involved in late apoptosis (Q2 area) were 9.81%. The amount of necrotic cells (region Q1) was 1.95% (Figure 4).

## 3.5 The results of the molecular method to quantitatively investigate the expression of Bax, Bcl-2, P53, TNF-a, and CASP3 genes in A549 cells treated with drugs using the qrt-PCR method.

### 3.5.1 Quantitative and qualitative assessment of RNA extraction

After RNA extraction, their quantitative and qualitative analysis was done using nanodrop and electrophoresis methods. Their concentration was read by nanodrop at wavelengths of 260 and 280 nm and its ratio was also calculated. Finally, the samples with an absorption rate higher than 1.8 were used to make cDNA. Also, RNA quality analysis using electrophoresis and % agarose gel. 1 was performed and then the 18s and 28s double bands were observed (Figure 5).

### 3.5.2 cDNA synthesis and primer setup

The results obtained from the prepared primers and synthesized cDNA from treated and untreated samples and the size of purchased primers were checked with Genome through Blast software and finally, PCR was performed. Also, at this stage, the best-hybridized temperature of the primer and the samples was obtained, and finally, the PCR results on the 1% agarose gel that were checked are shown in Figure 6.

### 3.5.3 To evaluate the relative quantitative Real-time PCR

To investigate the effect of the 5fu drug on the expression pattern of genes related to apoptosis in cancer cells, we measured the expression level of Bax, Bcl-2, P53, TNF-a, and CASP3 genes using quantitative Real-Time PCR method.

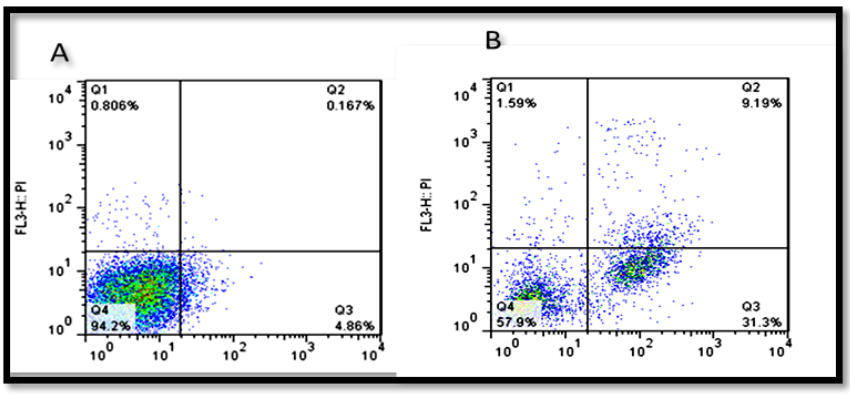


**Figure 4.** Flow cytometry analysis of A549 cell line. a) untreated control samples and b) drug-treated samples. Lower left square: viable cells, upper left square: early apoptosis, lower right square: necrosis, upper right square: delayed apoptosis. In drug-treated samples, cells enter early apoptosis and delayed apoptosis.

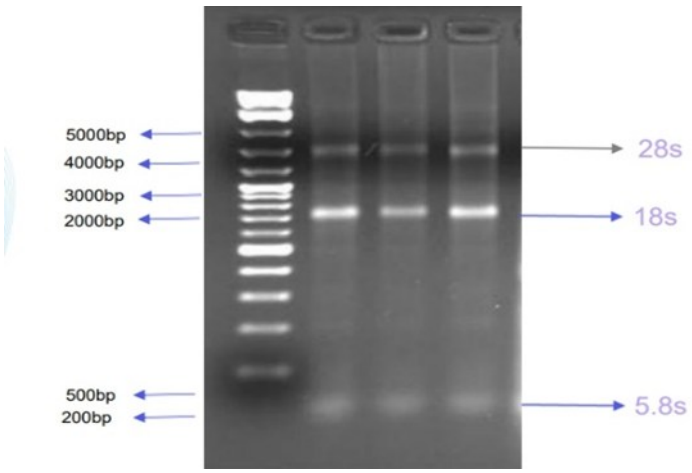


**Figure 5.** The image shows the results of the RNA quality assessment performed using gel electrophoresis. The gel displays distinct bands corresponding to the 28S and 18S ribosomal RNA (rRNA) subunits, which are commonly used as indicators of RNA integrity. The presence of these clear 28S and 18S rRNA bands suggests that the extracted RNA samples are of good quality and suitable for further analysis, such as cDNA synthesis and PCR.

3The results indicate that in the group treated with 5-fu drug, TNFa gene expression did not increase significantly. ($P < 0.05$). BAX gene expression increased significantly in the treatment group compared to the control group ($P<0.01$). These results show that 5-fu induces apoptosis in treated cells (figure 7).

### 3.5.4 Melting curve

After the completion of the PCR reaction, with a gradual increase in temperature, the double strands of DNA are separated and the fluorescence decreases. The resulting graph shows the melting temperature of the PCR product and the presence of a sharp peak indicates the specificity of the product (Figure 8).

## 3.6 The results of evaluating the expression of miRNA-142 and miRNA-195 genes on A549 cells treated with 5-fluorouracil using qRT-PCR method

### 3.6.1 The results of miRNA-142 gene investigation

The result of the analysis shows that the expression of the miR-142 gene is significantly increased. It's normalized to U6 and compared with the control gene(untreated) (Figure 9).

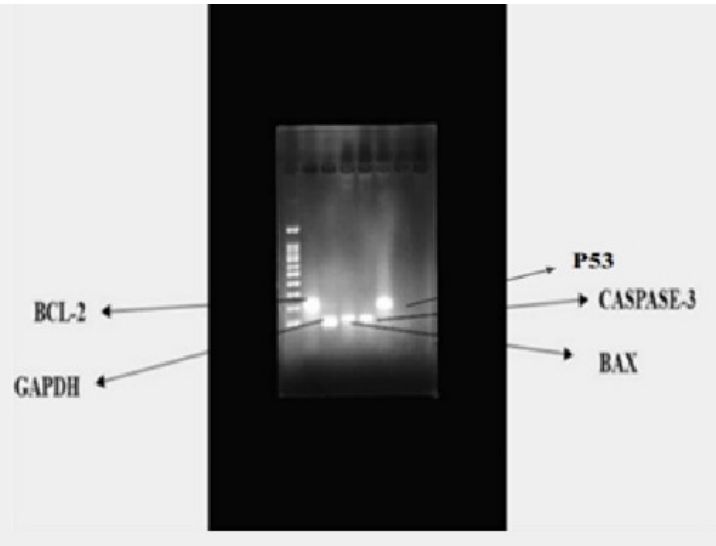


**Figure 6.** The image shows the results of a PCR analysis performed on treated and untreated samples to investigate the expression of miRNA-142. The gel electrophoresis image displays the amplified bands for different genes, including BCL2, GAPDH, CASPASE-3, and BAX. The treated and untreated samples are compared, allowing for the analysis of the relative expression levels of these genes between the two conditions.

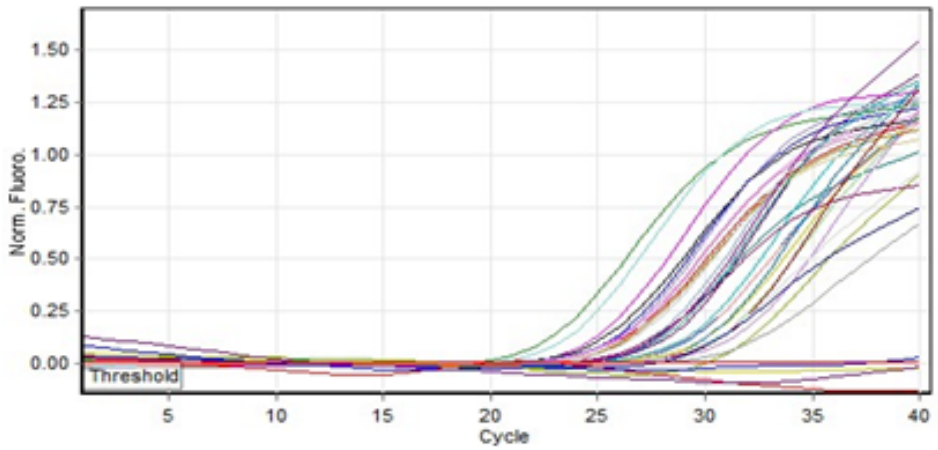


**Figure 7.** Gene duplication diagram A: GapDH, B: BAX, C: BCL-2, D: Caspase3

### 3.6.1 The results of miRNA-195 gene investigation

The expression of miR-195 is equal to 1.542, which indicates the increase of miR-195 expression. However, the p-value for the alternative hypothesis is equal to 0.169, which indicates that the increase in miR-195 gene expression is not statistically significant. Therefore, although the expression of the miR-195 gene increases, this increase is not statistically significant. It's normalized to U6 and compared with the control gene(untreated) (Figure 10).

### 3.6.3 The results of TNF-a gene investigation

The expression of TNF-a is equal to 0.753, which indicates the decrease of TNF-a expression. However, the p-value for the alternative hypothesis is equal to 0.167, which indicates that the decrease in TNF-a gene expression is not statistically significant. Therefore, although the expression of the TNF-a gene decreased, this decrease is not statistically significant. It's normalized to GAPDH and compared with the control gene(untreated) (Figure 11).

### 3.6.4 The results of miRNA-195 gene investigation

The results indicate that the expression of p53 is significantly increased in the treated sample compared to the control (untreated) sample. The relative gene expression value for the treated sample is approximately 4.5, which is a substantial increase compared to the control sample. This suggests that the treatment has led to a significant upregulation of the p53 gene (Fig 12).

### 3.6.5 BCL-2 gene investigation results

The results indicate that the expression of BCL-2 is significantly decreased in the treated sample compared to the control (untreated) sample, with a relative gene expression value of 0.553. This suggests that the treatment has led to a substantial downregulation of the BCL-2 gene (Fig 13).

### 3.6.6 BAX gene examination results

The results indicate that the expression of BAX is significantly increased in the treated sample compared to the control (untreated) sample. This suggests that the treatment has led to an upregulation of the BAX gene, which is known to play a role in apoptosis and cell death pathways (figure 14).

### 3.6.7 Caspase gene expression results

The results indicate that the expression of Caspase 3 is significantly increased in the treated sample compared to the control (untreated) sample. This suggests that the treatment has led to an upregulation of the Caspase 3 gene, which is a key mediator of apoptosis and programmed cell death (Figure 15).

## 4. Discussion

Non-small cell lung cancer (NSCLC) is considered the main subtype of lung cancer, accounting for 85 -90% of all cases. Persistent cough, pain, and weight loss are common symptoms in patients with NSCLC. Despite great advances in surgery, adjuvant therapy, and stereotactic radiotherapy, the 5-year overall survival (OS) rate of NSCLC patients is very poor (29).

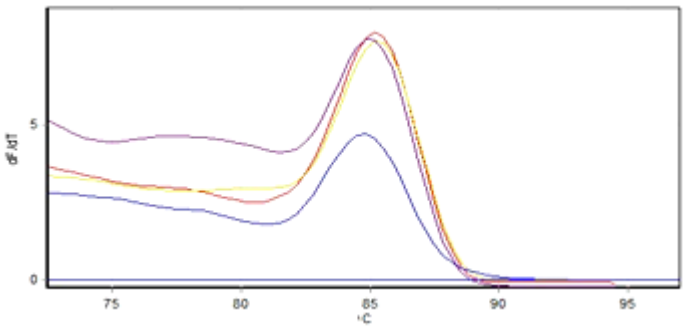


**Figure 8.** Determining the specificity of the Real Time PCR reaction using the melting curve A: GapDH, B: BAX, C: BCL-2 D: Caspase3

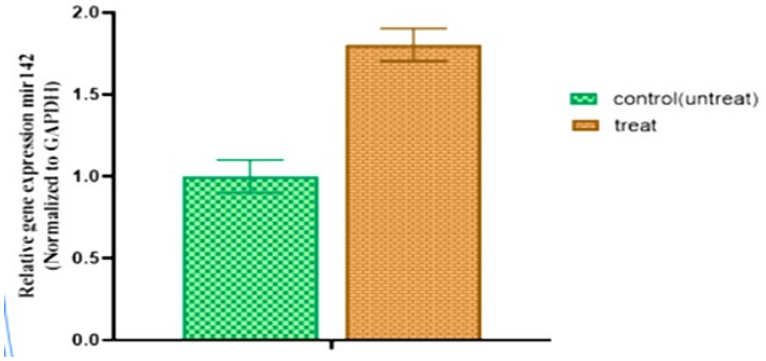


**Figure 9.** The figure shows the relative expression levels of the miRNA-142 gene in control (untreated) and treated samples. The green bar represents the control (untreated) group, while the orange bar represents the treated group. The data is normalized to the U6 control gene and compared to the untreated control group

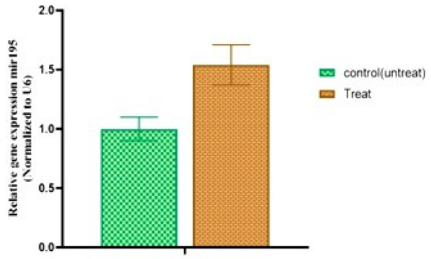


**Figure 10.** The image shows the relative gene expression of miRNA-195 in control (untreated) and treated samples, normalized to the U6 small nuclear RNA.

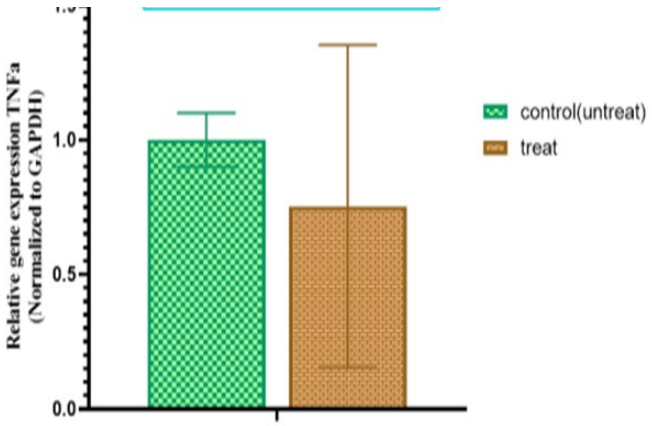


**Figure 11.** The image shows the relative gene expression of TNF-α in control (untreated) and treated samples, normalized to the GAPDH housekeeping gene. The results indicate that the expression of TNF-α is decreased in the treated sample compared to the control (untreated) sample, with a relative gene expression value of 0.753

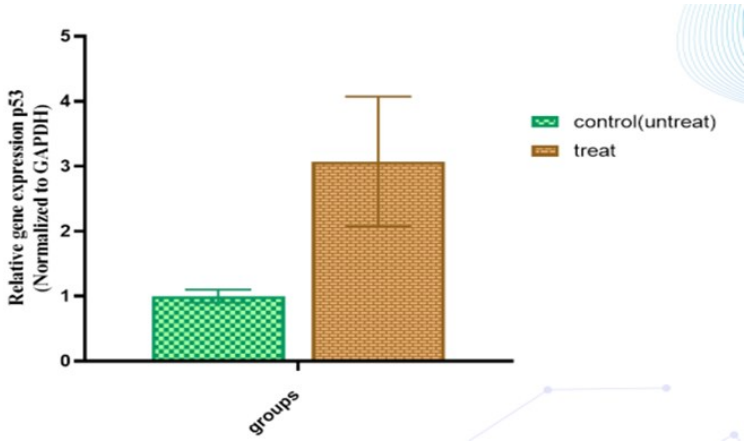


**Figure 12.** The image shows the relative gene expression of p53 in control (untreated) and treated samples, nor-

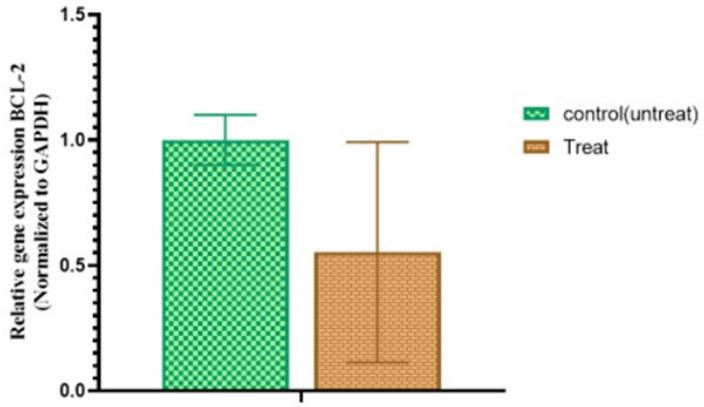


**Figure 13.** The image shows the relative gene expression of p53 in control (untreated) and treated samples, nor-

Therefore, optimization of current treatment methods requires a deep understanding of NSCLC pathogenesis.

MicroRNAs are short non-coding RNAs (20-23 nucleotides) that are commonly expressed in all types of tissues and cells and mediate post-transcriptional gene silencing in mammals through interaction with target mRNAs (30). Accumulating reports on the biological behaviors of miRNAs in development, proliferation, apoptosis, and differentiation have increased scientists' awareness of the important element of miRNAs in the pathophysiology of human diseases, including cancer(31).

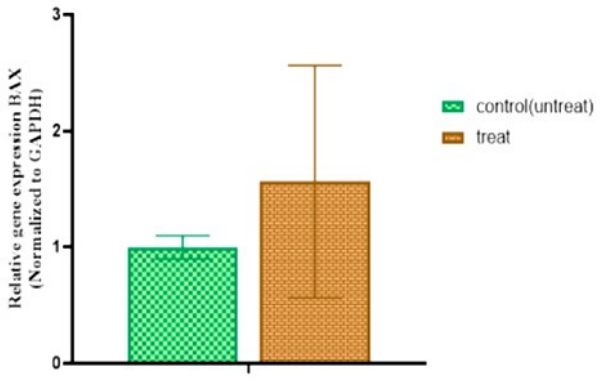


**Figure 14.** The image shows the relative gene expression of BAX in control (untreated) and treated samples, normalized to the GAPDH housekeeping gene.

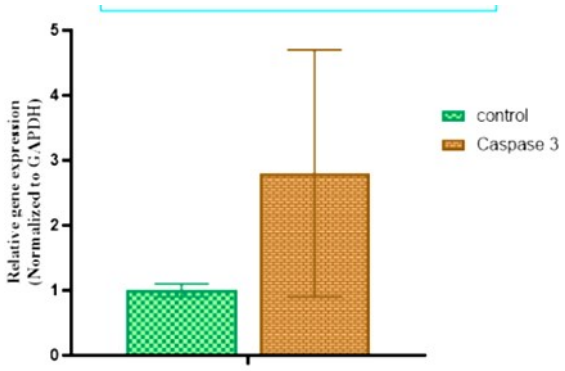


**Figure 15.** The image shows the relative gene expression of Caspase 3 in control (untreated) and treated samples, normalized to the GAPDH housekeeping gene.

miR-195 has been reported to suppress NSCLC tumorigenesis by modulating cyclin D3 and survival (32). However, the measurement and clinical significance of miR-195-5p in NSCLC remain undefined.

Among all miRNAs used as biomarkers, mir-142 plays an important role in modulating oncogenic molecules. Bioinformatics studies suggested the role of mir-142 in regulating the expression of key genes. A very striking finding regarding miR-142 and other miRNAs is that high miRNA levels were observed exclusively in the high-risk patient group (14).

Tumor suppressor p53 is a key regulator of apoptosis, and its activation leads to the upregulation of pro-apoptotic genes such as BAX and the downregulation of anti-apoptotic genes such as BCL-2. Interestingly, miRNA-142 and miRNA-195 regulate the expression of p53 and its target genes in NSCLC cells. Overexpression of miRNA-142 increases the expression of p53, BAX, and CASP3, while decreasing the expression of BCL-2 in cisplatin-treated NSCLC cells (33). Similarly, miRNA-195 overexpression increases the expression of p53, BAX and CASP3 and decreases BCL-2 in NSCLC cells (34). These findings suggest that miRNA-142 and miRNA-195 may sensitize NSCLC cells to chemotherapeutic agents, such as 5-fluorouracil, by modulating the expression of apoptosis-related genes.

This study that we have done can potentially help to formulate new treatment strategies and be effective in overcoming the problem of drug resistance in lung cancer. The aim of this study is to investigate the expression levels of miRNA-142 and miRNA-195 and their relationship with intrinsic and extrinsic apoptotic pathways in lung cancer cells treated with 5-fluorouracil.

A study focused on the development of a miR-129 mimetic modified with 5-fluorouracil (5-FU) as a potential therapeutic agent for non-small cell lung cancer (NSCLC). 5-FU-miR-129 significantly inhibited the proliferation of A549 and Calu-1 NSCLC cell lines. This indicates that the modified miRNA can effectively reduce the growth of cancer cells. Treatment with 5-FU-miR-129 resulted in increased apoptosis in NSCLC cell lines. This suggests that the modified miRNA not only stops cell growth but also induces programmed cell death, which is crucial for cancer treatment. Notably, the inhibitory effects of 5-FU-miR-129 can be achieved without the use of a delivery vehicle. This feature is important because it addresses a key hurdle in RNA-based therapies and makes it easier to deliver therapy directly to cancer cells (35).

Similar to our study, their results show the potential of 5-FU-miR-129 as a promising therapeutic option for NSCLC, especially in overcoming

resistance to existing therapies and providing a safer delivery method.

In another study, they presented a new approach by developing tumor suppressor microRNAs modified with 5-FU. This platform aims to increase the effectiveness of microRNA-based cancer treatments, potentially leading to improved treatment outcomes for cancer patients. One of the important findings is that 5-FU modification increases the stability of microRNAs in biological systems. This stability is important to ensure that therapeutic agents remain active in the body for longer, thereby increasing their effectiveness against cancer cells. This study shows that these modified microRNAs can be used in combination with existing cancer treatments, such as chemotherapy or targeted therapies. This combination can lead to synergistic effects, improving overall treatment efficacy and potentially reducing side effects (36).

A study by Hao Wang and colleagues investigated the role of miRNA-195-5p in non-small cell lung cancer (NSCLC) and its potential to reverse cisplatin resistance. miR-195-5p content was lower in cisplatin-resistant A549/DDP cells compared to A549 cells, suggesting an association between miR-195-5p levels and sensitivity to chemotherapy (37). FGF2 was identified as a target gene of miR-195-5p. This study showed that overexpression of FGF2 leads to increased resistance to cisplatin, while miR-195-5p can reverse this resistance by inhibiting FGF2. Similar to our study, this study provides evidence that miR-195-5p can enhance chemosensitivity to cisplatin in NSCLC by targeting FGF2, thereby providing potential therapeutic insights to overcome chemotherapy resistance. Presents in lung cancer.

The research team treated different miRNAs singly or in combination in A549 cells to measure their effect on the behavior of lung cancer cells, according to the authors of the article. In other words, they reduced the expression level of miRNAs in the studied cells regularly and stably to investigate its effect on the cancerous behavior of the cells. In a previous study, they identified miR-143 and miR-506, whose transient transfection induced apoptosis, cell cycle arrest, and decreased CDK1,4,6. In another study, similar to our study, they confirmed the stable changes of the expression of these miRNAs in A549 cells as a single and combined treatment on lung cancer cell behavior (38).

Similar to our study, various studies indicate that the expression of Bax, Bcl-2, p53, TNF-α, and CASP3 genes in A549 non-small cell lung cancer cells is indeed altered by 5-FU treatment. Research shows that 5-FU can induce apoptosis in A549 cells, mainly through modulation of the Bax/Bcl-2 ratio, which is critical for determining cell survival or death. Specifically, studies have shown that 5-FU treatment leads to upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl-2, promoting apoptosis through the caspase pathway, including CASP3 activation (39). Furthermore, the p53 pathway plays an important role in this process, as inhibition of MDM2 increases p53 activity and shifts cell death mechanisms more towards apoptosis in the presence of 5-FU(40).

## 5. Conclusion

The findings of this study provide valuable insights into the role of miRNA-142 and miRNA-195 in modulating apoptosis-related genes in non-small cell lung cancer (NSCLC) cells treated with 5-fluorouracil (5-FU). Treatment of A549 NSCLC cells with 5-FU resulted in a significant increase in the expression levels of miRNA-142 and miRNA-195 compared to the untreated control. This suggests these miRNAs may play an important role in the cellular response to 5-FU. The increased expression of miRNA-142 and miRNA-195 was accompanied by upregulation of pro-apoptotic genes such as p53, BAX, and Caspase-3 and downregulation of the anti-apoptotic gene BCL-2. This indicates that miRNA-142 and miRNA-195 can

sensitize NSCLC cells to 5-FU-induced apoptosis by modulating the expression of key genes in the intrinsic and extrinsic apoptotic pathways. Flow cytometry analysis confirmed that 5-FU treatment led to a significant increase in the percentage of A549 cells undergoing early and late apoptosis, further supporting the role of miRNA-142 and miRNA-195 in promoting apoptosis in response to 5-FU.

These findings suggest that miRNA-142 and miRNA-195 may be valuable biomarkers and therapeutic targets for improving the efficacy of 5-FU-based treatments in NSCLC. Further studies are warranted to fully elucidate the underlying mechanisms and explore the potential of these miRNAs in overcoming drug resistance in lung cancer.

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