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Molecular Study of the Impact of LDHA Gene and MIR-383 Interaction on the Progression of Ovarian Cancer in Iraqi Female Patients

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A B S T R A C T

Ovarian cancer is a major cause of death from gynecological malignancies globally, often detected at late stages due to non-specific symptoms, hence its nickname "silent killer." It arises from various ovarian tissues, mainly classified into epithelial, germ cell, and stromal tumors, with serous carcinoma as the most common form. Significant risk factors include genetic predispositions, such as BRCA1/BRCA2 mutations, reproductive history, and lifestyle factors, while socioeconomic differences markedly affect prognosis and treatment results. Recent studies underscore the significance of lactate dehydrogenase A (LDHA), a crucial enzyme in glycolysis; it promotes cell growth and metabolic changes that lead to the development of cancer. It is closely associated with hypoxia-inducible factor 1-alpha (HIF-1α). Furthermore, microRNAs, especially miR-383, are recognized as potential anti-tumor agents by targeting oncogenes like LDHA, influencing cancer cell activities including invasion, cell death, and chemotherapy resistance. The study aims to verify the inverse correlation between miR-383 and LDHA levels in ovarian cancer, highlighting their potential as therapeutic targets and markers for improving treatment strategies

Keywords: LDHA Gene, MIR-383, Ovarian Cancer

1. Introduction

Ovarian cancer is a type of cancer that originates in the ovaries, which are a component of the female reproductive system, is called ovarian cancer. It is frequently referred to as the "silent killer" because of its late diagnosis and vague symptoms. The most prevalent kinds are stromal cells, germ cells, and epithelial tumors. Genetic mutations (BRCA1 and BRCA2), age, family history, obesity, and reproductive history are risk factors for ovarian cancer. [1]. The symptoms include bloating, difficulty eating, frequent urination, changes in bowel habits, and abdominal pain. The diagnosis was made using imaging tests (CT, MRI, and ultrasound), blood tests (CA-125), physical examination, and biopsy.

Surgery, chemotherapy, immunotherapy, and targeted therapy are available treatments. The stage at diagnosis determines the prognosis; early detection increases survival rates[2].

One of the diverse types of neoplasms that is linked to mortality is ovarian carcinoma. One of the three primary ovarian components—the stroma, germinal cells, and epithelium—is classified as having OC due to its potential origin. As a result, ovarian cancers that are primarily malignant include Kruk Enberg's tumor, sex cord-stromal tumor, germ cell tumor, and epithelial carcinoma. Serous ovarian carcinoma is the most common, followed by epithelial carcinomas. However, the OC subtype differs depending on the age at which the appearance occurs. [3].

Ovarian cancer remains a significant public health concern with complex epidemiology and mortality on an international scale. In some regions, the incidence of ovarian cancer appears to be rising due to both genetic susceptibility and lifestyle factors. An increasing incidence and mortality of ovarian cancer in Korea has been observed, with some of the rise attributed to the westernization of lifestyles and demographic changes . Similarly, global estimates indicate 239,000 new cases of ovarian cancer and 152,000 deaths each year, making this disease one of the most aggressive gynecological malignancies [4]..Numerous risk factors contribute to the complex epidemiology of ovarian cancer. Furthermore, having a family history of breast or ovarian cancer is linked to an elevated risk (odds ratio = 3.7 for those with a personal history of breast cancer. Additionally, reproductive factors such as vulgarity, late age at first childbirth, and infant feeding practices have been associated with an increased risk of ovarian cancer. In contrast, the use of oral contraceptives is associated with a 30% to 50% reduction in the risk of ovarian cancer, highlighting the significant role of hormonal factors in the epidemiology of this disease[5].

The mortality rates associated with ovarian cancer are, of course, influenced by factors such as socioeconomic status and access to care. Studies indicate that neighborhood socioeconomic status (SES) affects patient outcomes in various solid tumors, including ovarian cancer, and that these associations differ by racial group . While Black women are less likely to develop breast cancer than white women, they experience higher mortality

rates from the disease highlighting significant healthcare disparities. Additionally, comorbidities, particularly cardiovascular disease (CVD), have been linked to poor treatment response and survival in women with ovarian cancer thereby contributing to the worsened outcomes for patients[6].

Ovarian cancer represents a significant public health challenge, largely due to delayed diagnoses and inadequate screening methods. More than 70% of women are diagnosed at advanced stages, which results in a five-year survival rate below 30%. Understanding the patterns of incidence and mortality, particularly among older women, is vital for combating this disease. Treatments such as chemotherapy are crucial for improving survival rates Genetics also play a pivotal role, as inherited mutations in the BRCA1 and BRCA2 genes substantially increase the risk of cancer. Genetic testing can identify individuals at risk and inform prevention strategies. Improving both screening and treatment is critical to reducing mortality rates[7].

Lactate dehydrogenase A, which is produced by the LDHA gene, located in the shorter arm of chromosome 11 is essential for glycolysis and is frequently overexpressed in a variety of cancers. Numerous transcription factors, such as c-Myc, Sp1, and CREB, as well as posttranscriptional mechanisms, regulate its expression. The overexpression of LDHA promotes cell proliferation, aerobic glycolysis, and the epithelial-mesenchymal transition, all of which contribute to the advancement of tumors[8].. Higher malignancy and poorer patient outcomes are linked to elevated LDHA levels in oral squamous cell carcinoma. LDHA and c-Myc have a well-established relationship, with c-Myc's activation of LDHA being essential for tumor growth and metabolism. Its potential as a target for cancer therapy is highlighted by the ability to inhibit tumor growth and malignancy by targeting LDHA expression or function. Through its role in aerobic glycolysis and interaction with hypoxia-inducible factor 1-alpha (HIF-1α), LDHA has a significant impact on the progression of ovarian cancer. The overexpression of LDHA and HIF-1α in ovarian cancer frequently results in a feedback loop that promotes tumor growth. Inhibiting LDHA with JQ1, a BET bromodomain inhibitor, can stop tumor growth by altering cancer metabolism, affecting c-Myc, and causing cell cycle arrest. Furthermore, LDHA interacts with the ras p21 protein in ovarian tissues, and many cases of ovarian cancer exhibit simultaneous overexpression of the ras and LDHA genes, suggesting a potential role in cell transformation and the advancement of the cancer [9].

MicroRNAs (miRNAs), a class of small non-coding RNAs, regulate gene expression by inhibiting translation or promoting degradation of target mRNAs. Such short non-coding RNA (18–24 nucleotides) molecules involve several biological processes, including apoptosis, development, and proliferation. Primary miRNAs are transcribed, processed via the action of Drosha and Dicer enzymes, and further matured to form functional miRNAs [10] . They are involved in many diseases, particularly cancer and neurodegenerative disorders. Similar tumor classification and prognostic signatures have also been achieved using miRNA expression profiles. One promising avenue of clinical exploration is using antagomirs, chemically modified single-stranded oligonucleotides that specifically silence target miRNAs in vivo for therapeutic applications. Moreover, artificial intronic miRNAs have been created for gene function experimentations and potential gene therapy[11].

MicroRNA-383 (miR-383) is a tumor suppressor in variety of cancers, such as ovarian cancer and non-small cell lung cancer (NSCLC), through inhibition of proliferation. It is often downregulated in cancerous tissues and cell lines, while its overexpression can inhibit the invasion, migration or proliferation of cancer cells. Downstream of miR-383 are multiple targets (CCND1, LDHA, VEGF, IGF and Gadd45g) that influence apoptosis, aerobic glycolysis, DNA repair, etc. it modulates pluripotent genes that influence the differentiation of embryonic stem cells as well (4, 5). MiR-383 may be a potential therapeutic target and prognostic biomarker in cancer treatment[12]. Despite the widespread deregulation of miR-383 across many cancers, and associations with features attributable to its role in various aspects of tumorigenesis such as cell cycle regulation, migration or proliferation, the precise functions and mechanisms regulated by miR-383 remain unclear. MiR-383 is considered a potential ovarian cancer tumor suppressor. According to studies, miR-383 inhibits the progression of ovarian cancer in a number of ways, including by reducing the growth of cancer cells by targeting genes that regulate the cell cycle and proliferation, increasing apoptosis in cancer cells by possibly modifying apoptotic factors, and possibly preventing metastasis by preventing cell migration and invasion. Furthermore, miR-383 may help overcome drug resistance by increasing the ovarian cancer cells' receptivity to chemotherapy[13].

MicroRNAs play a role in both the development of cancer and the control of LDHA gene expression. According to research, miR-383 directly targets LDHA to prevent cell invasion, proliferation, and glycolysis in ovarian and hepatocellular carcinoma. Similarly, miR-34a suppresses LDHA in breast cancer, which lowers glycolysis and cell proliferation and acts as a tumor suppressor[14]. A poor prognosis is indicated by low levels of miR-30d-5p, which targets LDHA in gallbladder cancer. LDHA promotes tumor growth, invasion, and aerobic glycolysis and is generally overexpressed in cancerous tissues relative to healthy ones. Cancer cell growth and invasion are inhibited when LDHA is suppressed by microRNA regulation or medication interventions, suggesting that the microRNA-LDHA pathway may be a useful target for cancer treatment. The goal of our current study is to prove that human ovarian cancer has decreased levels of miR-383. In ovarian cancer, miR-383 expression is inversely correlated with LDHA levels, and aberrant expression of miR-383 inhibits cell growth and invasion by directly targeting LDHA. Therefore, a key factor in the development of ovarian cancer is the miR-383/LDHA axis [15].

2. Methods and Materials

This research was conducted between August 2023 and January 2024. Every study experiment was carried out at the Iraqi Hereditary Company (IHC) and the University of Technology.

2.1. Study Group

One hundred and forty people participated in the study. Samples were drawn at random based on the number of patients present; the following study groups were included:

Group 1: For a private laboratory in Baghdad, Iraq, 70 samples of women between the ages of 30 and 70 who appeared to be in good health were collected.

Group 2: Al-Amel Hospital in Baghdad, Iraq, received samples from 70 Iraqi women between the ages of 30 and 70 who had been diagnosed with ovarian cancer.

2.2. Sampling of Blood

Each participant's venous blood had to have two milliliters of whole blood extracted under sterile conditions and put straight into a tube that contained EDTA.

2.3. Total RNA Extraction with Trizol

RNA was extracted from the whole blood of both patients and healthy controls using TRIzol with the EasyPure® TransZol Up Plus RNA Kit (Cat. No.: ER501) protocol. After sample collection, TRIzol Lysis Reagent was applied, followed by chloroform to separate the aqueous phase. The samples were then vortexed, incubated at room temperature for 3 minutes, and centrifuged at 10,000 rpm for 15 minutes. Ethanol was introduced to the aqueous phase to ensure proper binding of all RNA molecules. Finally, the samples were eluted in RNase-free water and stored at -20°C for subsequent analysis.

2.4. MicroRNA extraction using whole blood

Using the EasyPure® miRNA kit protocol (Cat. No.: ER601), miRNA is extracted from the whole blood of both patients and healthy controls. After adding Lysis Buffer 10 (LB10) to the blood mixture, it is allowed to sit at room temperature for five minutes. After that, chloroform is added to the mixture. After three minutes of vigorous hand shaking at room temperature, the tube is centrifuged for fifteen minutes at 10,000 rpm. Ethanol is added to the resultant aqueous phase to guarantee that all miRNA molecules bind as best they can.

2.5. The primers

The NCBI (National Center Biotechnology Information) bioinformatics program was used to obtain the primers designed in this study. For both forward and reverse primers, 10 picomol was the ideal concentration. Table 1 investigation along with their sequences.

Table 1. lists the primers used in this study

Primers	Sq. (5′→3′ direction)
LDHA Human qPCR expression Primer	
F	TGTGCCTGTATGGAGTGGAA
R	AGCACTCTCAACCACCTGCT
GAPDH Human qPCR expression	
F	TGAGAAGTATGACAACAGCC
R	TCCTTCCACGATACCAAAG
miRNA	
Mir-383 F.	AGATCAGAAGGTGATTGTGGCT
miRU6 F.P.	AGAGAAGATTAGCATGGCCCCT
universal R. transcription p	CAGGTCCAGTTTTTTTTTTTTTTVN
miRNA-universe R.P.	GCGAGCACAGAATTAATACGAC

2.6. qRT-PCR (real-time PCR)

The Stratagene Real-time PCR System (Analytik Jena Technologies) with qPCR soft software was used to perform qRT-PCR. Gene polymorphism was measured using the probe color reaction and the components of the 2xqPCR Master Mix Kits. Each reaction had three duplicate negative controls: a non-template control (NTC), a non-amplification control (NAC), and a non-primer control (NPC). The 2-ΔCt method was used to calculate the expression ratio without a calibrator sample[16] .

$$\Delta CT\ (test) = CT\ gene\ of\ interest\ (target, test) - CT\ internal\ control$$

2.7. The qPCR Reaction

2.7.1 LDHA gene Amplification:-

The expression of the LDHA gene was measured using real-time PCR (RTq-PCR) in accordance with the thermal profile displayed in Table 2.

Table 2. Thermal profile of LDHA gene expression.

Step	Temperature	Duration	Cycles
Enzyme activation	94ºC	30 sec	Hold
Denature	94 ºC	5 sec	40
Anneal	58 ºC	15 sec	
Extend	72 ºC	15 sec	

2.7.2 Amplification of miR-383

The EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, China) kit was used to reverse transcribe the expression for cDNA synthesis in accordance with the manufacturer's instructions. Following the manufacturer's instructions, RTq PCR analysis was performed using the TransStart® Top Green qPCR SuperMix (Transgen, China) to quantify the expression. Table 3 displays the conditions for the cDNA reverse transcription heat cycler steps.

Table 3: The conditions for the heat cycler steps of cDNA reverse transcription.

	Step1	Step2	Step3
Temperature	25°c	42°c	85°c
Time	10min	15min	5seconds
Reaction	Random Primer (N9) binding	Anchored Oligo(dT)18 binding	Inactivate reverse transcriptase enzym

2.8. Housekeeping Gene Amplification

Using the housekeeping gene, the ΔCT value was determined using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control [23]. A qPCR experiment was conducted to amplify GAPDH using the supplied thermal profile (Table 4).

Table 4: GAPDH gene expression thermal profile.

Step	Temperature	Duration	Cycles
Enzyme activation	94ºC	30 sec	Hold
Denature	94 ºC	5 sec	
Anneal	58 ºC	15 sec	40
Extend	72 ºC	15 sec	

2.9. Analysis of statistics

The system for statistical analysis was employed (SAS, 2012). The analysis of variance (ANOVA) method's least significant difference (LSD) test was employed to compare means in a meaningful way. The chi-square test was used to compare percentages significantly at the 0.05 and 0.01 probability levels. The correlation coefficient between the variables in this study was calculated.

3. Results and Discussion

The GAPDH gene and the ovarian cancer group were investigated using a quantitative RT-PCR technique. Table 5 shows the housekeeping gene used in this study, GAPDH. In the healthy group, the GAPDH Ct value ranged from 17.21 to 18.15, with a mean ± SD of 17.21 to 0.56. The ovarian cancer group's mean ± SD was 17.79 ± 0.46, with a range of 17.89-18.11. With an LSD value of 0.185, a non-significant difference in the mean Ct value of GAPDH between these groups was observed ($p = 0.921$: $p<0.05$).

Table 5: Comparison of the GAPDH Ct value (mean±SD) for the various groups under study.

Group	No.	Mean ± SD of Ct value	Range
Group 1 Control	70	17.21 ±0.56 .	17.21-18.15
Group 2 ovarian cancer group	70	17.79 ±0.46	17.89-18.11
LSD	--	0.185 NS.	---
P-value	--	0.921	---

NS: Non-Significantly.

The ovarian cancer group's 2^-ΔCt value was 2.55189E-06, while the healthy group's was 2.51153E-06. For the healthy group, the gene expression fold change ratio was 1.00 ± 0.00, while for the ovarian cancer group, it was 1.01607 ± 0.04. These findings are shown in Table 6.

Table 6: GAPDH Fold Expression Comparison of the Ovarian Cancer and Control Groups

Group	Means Ct of GAPDH	2-Ct	Experimental group/ Control group	Fold of gene expression
Group 1 breast cancer	18.58	2.55189E-06	2.55189E-06/2.51153E-06	1.01607± 0.04
Group 2 control	18.603	2.51153E-06	2.51153E-06/2.51153E-06	1.00 ± 0.00

Our results are consistent with those of R. Barber et al., which show that GAPDH, often used as a housekeeping gene for gene expression reference because of its assumed constant expression across various tissues, has stable mRNA levels[17]. In a comprehensive study, researchers evaluated GAPDH mRNA levels in 72 distinct healthy human tissues, using 371,088 real-time RT-PCR measurements. The findings indicated significant uniformity in GAPDH mRNA expression, both across different tissue types and within individuals of the same tissue type[18].

The

groups	Means of Ct LDHA	Means of Ct GAPDH	ΔCt (Means of Ct LDHA - Means Ct of GAPDH)	2-ΔCt	experimental group/ Control group	Fold of gene expression
Group ovarian cancer group	23.56	18.58	4.98	0.031686	0.031686/0.006185	5.123039
Group 2 control	25.94	18.603	7.337	0.006185	0.006185/ 0.006185	1.00

calculated LDHA expression ratio in the ovarian cancer group was 5.123039, compared to 1.00 in the control groups. The results indicated that LDHA expression was significantly higher in the ovarian cancer group than in the control group. Additionally, it was observed that the average expression level of LDHA was elevated across various cancer groups, with the mean CT values for the ovarian cancer group being 23.56, which were lower than those of the control group at 25.94. (table 7)

Table (7): Fold of LDHA expression Depending on 2- ΔCtMethod

Our findings are consistent with those of Wei et al., where a meta-analysis of 2,182 patients showed that high serum LDH levels were significantly associated with reduced overall survival (OS), with a hazard ratio of 1.86, signifying its strong prognostic value . Similarly, our results concur with Mireștean et al., [19] who found that elevated LDH levels were connected to poor prognosis and treatment discontinuation, underscoring its role in tumor proliferation and necrosis. This is in line with findings by Shaaban & Taalab, [20] where in AML patients, high LDH levels were linked to shorter OS, reinforcing its status as a predictive marker for survival outcomes [21]

Lactate dehydrogenase A (LDHA) plays a pivotal role in cancer metabolism, particularly through its facilitation of aerobic glycolysis, which is crucial for tumor growth and survival. Elevated LDHA levels are associated with poor prognoses in various cancers, including ovarian and pancreatic cancers, where its overexpression promotes lactate production, thereby enhancing glycolytic flux and altering the tumor microenvironment. In ovarian cancer, LDHA not only supports tumor metabolism but also influences immune responses, as lactate can modulate immune signaling pathways(Sun et al., 2024) [22] [23] Inhibition of LDHA has been shown to disrupt energy supply in cancer cells, induce apoptosis, and impair immune cell recruitment, highlighting its dual role in tumor progression and immune evasion. Furthermore, LDHA's interaction with signaling molecules like Rac1 suggests that its oncogenic effects extend beyond metabolic functions, indicating a complex regulatory network that could be targeted for therapeutic interventions. However, careful consideration is necessary when targeting LDHA to avoid detrimental effects on normal cellular metabolism and immune function [24]

The Comparison between ovarian cancer and control groups groups in Ct value of miRU6 the housekeeping gene in the present study, is shown in Table 8. The healthy group The (control group) had a Ct value for miRU6 that ranged from 29.24-31.59 (30.14±0.35). In the ovarian group , it varied between 28.10and 30.41 (29.4±0.14). The mean Ct value of miRU6 did not differ significantly between these groups (P = 0.892). Housekeeping genes are employed in molecular studies based on the assumption that their expression remains constant in the cells or tissues under investigation. miRU6 is one of the most commonly utilized housekeeping genes for comparing gene expression data..

Table (8) Comparison between different groups in Ct value of miRU6 (Mean±SD).

Group	No.	Mean±SD of Ct value	Range
Group1 ovarian cancer	70	29.4±0.14	28.10-30.41
Group 2 control	70	30.14±0.35	29.24-31.59
P-value		0.892	

Our findings indicate no significant expression changes of miRU6 across the study groups, with fold changes of 0.991 and 1.00 in the ovarian cancer and control groups, respectively (Table 9) . These results are consistent with those reported by Duan et al., confirming that U6 is a trustworthy reference gene for normalizing miRNA data in urinary sediment studies linked to IgA nephropathy. Furthermore, our findings support those of Causin et al. by showing that U6 is among the most stable housekeeping genes in liquid-based cervical cytology samples, improving the precision of miRNA quantification. [25] [26]

Group	Means Ct of miRU6	2-Ct	experimental group/ Control group	Fold of gene expression
Group 1ovarin cancer	29.4	1.41162E-09	1.41162E-09/8.68955E-10	1.62
Group 2 control	30.1	8.68955E-10	8.68955E-10/8.68955E-10	1.00

Table(9) Comparison of miRU6 Fold expression between study groups

Expression of mir-383 was examine by A quantitative RT-PCR technique depending on 2-ΔCt method .

The fold of ovarian cancer group was 0.570382 while to that of the control group was 1.00 (Table 10).

Table (10): Fold of miR-383 expression depending on 2-ΔCt method.

Groups	Means Ct of miR-383	Means Ct of miRU6	ΔCt (Means Ct of miR383 - Means Ct of miRU6)	2-ΔCt	experimental group/ Control group	Fold of gene expression

Group 1ovarain cancer	28.18	29.4	-1.22	2.329467	2.329467/4.084049	0.570382
Group 2 control	27.97	30.1	-2.03	4.084049	4.084049/ 4.084049	1.00

The study sheds light on a mechanism where miR-383 targets LDHA in ovarian cancer cells. It attaches to the 3'-untranslated region of LDHA, which leads to reduced expression [27] [28] . This reduction hinders the proliferation and invasion of the cancer cells. Additionally, this interaction impacts aerobic glycolysis within the cells, highlighting miR-383's role in controlling cancer metabolism and its progression[29] [30] . miR-383 is pivotal in ovarian cancer by curtailing the spread and growth of cancer cells. By targeting LDHA, it influences aerobic glycolysis in these cells. The research suggests that miR-383 may be a potential diagnostic biomarker and a therapeutic target for treating ovarian cancer. [31] [32]

5. Conclusions

The correlation between diminished miR-383 levels and elevated LDHA gene expression in ovarian cancer underscores a promising biomarker for tracking cancer progression and devising treatment plans. It accentuates the necessity for additional studies to investigate their interplay and the consequent effects on patient prognosis. Comprehending this relationship may lead to ground-breaking treatments aimed at these molecular elements..

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Conflict of Interest

There is no conflict of interest, according to the authors.

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