T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF MOLECULAR MEDICINE

INVESTIGATION OF THE EFFECTS OF MIRNA- 582-5P AND MIRNA-363 EXPRESSION LEVELS ON CASPASE-9 IN GLIOBLASTOMA MULTIFORME TUMORS

MASTER OF SCIENCE THESIS

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ISTANBUL-2019

THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Programme : Master's Program in Molecular Medicine

Title of the Thesis : Investigation of the Effect of miRNA-582-5p and miRNA-363 Expression Levels on Caspase-9 in Glioblastoma Multiforme Tumors

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Examination Date : 28/06/2019

This study have approved as a Master Thesis in regard to content and quality by the Jury.

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

28.06.2019

Deryanaz BILLUR

DEDICATION

I dedicate to my thesis

to my sainted grandmother, Semiha Izgi

and

to my lovely Family

ACKNOWLEDGEMENTS

Firstly, I would like to express my special gratitude and thanks to my thesis supervisor Prof. Dr. Turgay ISBIR, for his immense knowledge. His excellent guidance and support on my research is priceless.

Secondly, I would like to thank Cumhur Kaan YALTIRIK, M.D. and Selcuk OZDOGAN, M.D. for their support on my research during my master.

My sincere thanks also go to Molecular Medicine members; Assoc. Prof. Seda GULEC-YILMAZ, Muge KOPUZ-ALVAREZ NOVAL, PhD., Selvi DUMAN-BAKIREZER, PhD., Huseyin AYHAN, MSc. and Emre Murat ALTINKILIC, MSc. for providing me with scientific environment.

Also, I thank to my friends and colleagues Ipek Yagmur ABAOGLU, Burak Gizem GOLES and Ozum Begum BOKE for sharing friendly atmosphere.

I wish to special thanks to my good friend David Sinan ESENSOY for his endless support even though his full medical school lecture schedule.

I wish to give my heartfelt thanks to my lovely parents Iffet IZGI-BILLUR and Resul BILLUR for their endless support during my good and bad times.

Deryanaz BILLUR

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$\Delta\Delta C_T$	: Delta Delta C_T
ΔC_T	: Delta C_T
AGO2	: Argonaute 2
AIF	: Apoptosis Inducing Factor
Apaf-1	: Apoptotic Protease Activating Factor 1
AUC	: Area Under Curve
Bak	: Bcl-2 Antagonist/killer-1
Bax	: Bcl-2-Associated X Protein
BCNU	: Carmustine
C.I	: Confidence Interval
CARD	: Caspase Activation Domain
CCNU	: Lomustine
cDNA	: Complementary DNA
CNS	: Central Nervous System
CT	: Computed Tomography
C_T	: Cycle Threshold
Cyt C	: Cytochrome C
DGCR8	: DiGeorge Syndrome Critical Region-8
DIABLO	: Direct Inhibitor of Apoptosis Protein-Binding Protein with Low PI
DISC	: Death Inducing Signaling Complex
DNA	: Deoxyribonucleic Acid
DR	: Death Receptor
EGFR	: Epidermal Growth Factor Receptor
FADD	: Fas-Associated Death Domain
GBM	: Glioblastoma Multiforme
IAP	: Inhibitor of Apoptosis Protein
IDH-1	: Cytosolic $NADP^+$ related isocitrate dehydrogenase
$\ln(2^{-(\Delta\Delta CT)})$	: Fold change
LNA	: Locked nucleic acid

IncRNA : Long noncoding RNA

LOH : Loss of Heterozygosity

µl : microliter

m^7G : 7-methylguanosine

MDM2 : Mouse Double Mnute 2

mg : milligram

miRNA : Micro RNA

MRI : Magnetic Resonance Imaging

mRNA : Messenger RNA

NADP+ : Nicotinamid Adenin Dinucleotide Phosphate

ng : Nanogram

OD : Optic Dencity

OR : Odds Ratio

PAN2 : Poli(A)-nuclease 2

PAN3 : Poli(A)-nuclease 3

PCR : Polymerase Chain Reaction

PDGFA : Platelet-Derived Growth Factor Receptor Alpha

pg : picogram

piRNA : Interacting RNA

pre-miRNA : Precursor-miRNA

pri-miRNA : Primer-miRNA

PTEN : Phosphate and Tensin Homolog

RB : Retinoblastoma

RISC : RNA Induced Silencing Complex

RNA : Ribonucleic Acid

RNase III : Ribonuclease III

ROC : Receiver Operating Characteristic

rpm : Rounds per minute

RT-PCR : Real-Time Polymerase Chain Reaction

SD : Standard deviation

shRNA : Short hairpin RNA

siRNA : Small Interfering RNA

SMAC : Second Mitochondria-Derived Activator of Caspase

SNP : Single Nucleotide Polymorphism

TMZ : Temozolomide

TNF : Tumor Necrosis Factor

TRAIL : Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

TRBP : Transactivating Response RNA Binding Protein

UTR : Untranslated Region

WHO : World Health Organization

XPO5 : Exportin 5

XRN1 : Exonuclease-1

ABSTRACT

Billur, D. Investigation of the Effect of miRNA-582-5p and miRNA-363 Expression Levels on Caspase-9 in Glioblastoma Multiforme Tumors. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine. Master Thesis. Istanbul, 2019.

The aim of this thesis study is to investigate the expression levels of microRNA-582-5p and miRNA-363 as they relate to serum levels of caspase-9, which holds key roles in the initiation of apoptosis in glioblastoma multiforme (GBM) patients. For this purpose, a total of 71 individuals, were selected and divided into two groups: those who were diagnosed with GBM disease (n=35), and those who were not previously diagnosed with this disease (n=36).

The expression levels of miRNA-582-5p, miRNA-363 and caspase-9 gene Ex5+32 G>A (rs1052576) polymorphism of the groups were analyzed using real-time polymerase chain reaction. The miRNA expression analysis showed that miRNA-582-5p and miRNA-363 expressions were significantly downregulated in the patient group (***p*=0.014*** and ***p*=0.010***). When the means of serum miRNA-582-5p, miRNA-363 and serum caspase-9 levels were compared, a statistically significant relationship between miRNA expression levels and caspase-9 level could not be determined (*p*=0.144 and *p*=0.050). Also, caspase-9 gene Ex5+32 G>A (rs1052576) polymorphism was investigated in GBM patients, as well as undiagnosed individuals in the Turkish population. Having GG genotype reduced the risk of GBM by 3.78 times (***p*=0.015***). According to the results of ROC analysis, which is the last assessment point for biomarker nomination, it was observed that miRNA-582-5p and miRNA-363 are candidate biomarkers for GBM (miRNA-582-5p ΔCT ***p*=0.006***; fold change ***p*= 0.0001*** and miRNA-363 ΔCT ***p*=0.0016***; fold change; ***p*=0.0001***).

In conclusion, while a statistically significant relationship was found between miRNA-582-5p, miRNA-363 expression levels and working groups, no statistically significant relationship was found between miRNA-582-5p and miRNA-363 expression levels regarding an effect on serum caspase-9 levels.

***Key Words*:** Glioblastoma Multiforme, miRNA-582-5p, miRNA-363, Caspase-9

ÖZET

Billur, D. Glioblastoma Multiforme Tümörlerinde miRNA-582-5p ve miRNA-363 Ekspresyon Seviyelerinin Kaspaz-9 Üzerine Etkisi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı. Yüksek Lisans Tezi. İstanbul, 2019.

Bu tezin amacı, miRNA-582-5p ve miRNA-363 ekspresyon seviyelerinin, apoptozun başlatılmasında kilit role sahip olan kaspaz-9'un serum düzeyinde ilişkisinin glioblastoma multiforme (GBM) hastalarında araştırılmasıdır. Bu amaçla, toplam olarak 71 birey seçilerek, GBM tanısı almış olan (n=35) ve daha önce bu hastalığın tanısını almamış olanlar (n=36) olmak üzere iki gruba ayrılmıştır.

Gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak grupların miRNA-582-5p, miRNA-363 ekspresyon seviyeleri ve kaspaz-9 geni Ex5+32 G>A (rs1052576) polimorfizmi analiz edilmiştir. miRNA ekspresyon analizleri, miRNA-582-5p ve miRNA-363 ekspresyon seviyelerinin anlamlı olarak hasta grubunda anlatımının azaldığını göstermiştir (***p*=0.014*** ve ***p*=0.010***). Serum miRNA-582-5p, miRNA-363 ve serum kaspaz-9 seviyeleri karşılaştırıldığında, istatistiksel olarak miRNA ekspresyon seviyesi arasında bir anlamlılık tespit edilememiştir (*p*=0.144 ve *p*=0.050). Ayrıca, Türk populasyonunda kaspaz-9 geni Ex5+32 G>A (rs1052576) polimorfizmi GBM hastalarının yanı sıra tanı konulmamış bireylerde araştırılmıştır. GG genotipine sahip olmak, GBM riskini 3.78 kat azaltmaktadır (***p*=0.015***). Biyobelirteç adaylığının son değerlendirme noktası olan ROC analiz sonuçlarına göre, miRNA-582-5p ve miRNA-363'ün GBM için biyobelirteç adayı olabileceği gözlemlenmiştir (miRNA-582-5p ΔCT ***p*=0.006***; kat değişimi ***p*=0.0001*** ve miRNA-363 ΔCT ***p*=0.0016***; kat değişimi ***p*=0.0001***).

Sonuç olarak, çalışma grupları ile miRNA-582-5p ve miRNA-363 ekspresyon seviyeleri arasında istatistiksel olarak anlamlı bir ilişki bulunmuş olup, miRNA-582-5p ve miRNA-363 ekspresyon seviyelerinin serum kaspaz-9 seviyesi üzerinde bir etkisi olmadığı bulunmuştur.

***Anahtar Kelimeler*:** Glioblastoma Multiforme, miRNA-582-5p, miRNA-363, Kaspaz-9

1. INTRODUCTION AND PURPOSE

Glioblastoma multiforme (GBM) is a brain tumor with a high level of destruction that ends in a short period of 14 months (1).

According to statistical studies from the United States of America, the rate of death caused by malignant brain tumors is 22,000 cases per year, and approximately 80% of these cases are glioblastoma-induced (2). For the treatment of the disease following surgical operation, clinical applications such as radiation and chemotherapy are performed in a series (3).

MicroRNAs (miRNA) are small, non-coding RNA molecules that are involved in many vital processes. The miRNAs, which can be encoded both inter- and intra-genically, are clipped from long RNAs. Firstly the microRNAs, which can be processed as pre-miRNA, pass into the cytoplasm and become a double-stranded miRNA of about 22 nucleotides in length. One of the two chains of miRNA interacts with the protein complex called RNA Induced Silencing Complex (RISC). The ribonucleoprotein complex, which is the result of this interaction, prevents expression of messenger RNA (mRNA) by binding to them. Although this procedure is normal, it is reported that it is damaged in cancer cells (4).

Recent studies have shown that miRNAs play an active role in tumor survival and growth. Based on this information, different types of glioblastoma and healthy brain tissue have been studied and new microRNA expression models have been revealed (5-7).

Research on many types of cancers such as GBM has shown that miRNAs have a triggering effect on tumor development.

Some miRNAs have been reported to have oncogenic properties (8,9). In contrast to oncogenic miRNAs, tumor suppressor miRNAs have low expression in GBM and decrease in growth and invasion (10, 11).

The two first-rate-defined apoptotic pathways present in mammals-the extrinsic or receptor-brought about pathway and intrinsic or mitochondrial stress-brought about pathway—each use the caspase cascade.

The two apoptotic pathways determine the final physical status of cell systems via executioner proteases referred to as caspases, which are usually subdivided into initiator and effector caspases. Procaspases are synthesized as single-chain inactive zymogens and must be activated for apoptotic process.

Caspase-9 is the foremost molecule in the intrinsic or mitochondrial pathway that is triggered by various factors such as chemotherapies, cellular stress and radiation. In order to maintenance of catalytic activity of caspase 9, it is triggered on the apoptosome complex. In case of the defects of caspase-9, there are profound physiological and pathophysiological results which lead to degenerative problems and developmental problems such as cancer (12).

According to a research conducted in this context, miRNAs 582-5p and 363, which were reported as oncogenic miRNAs, that are the subject of this thesis, have been shown to inhibit apoptotic mechanism by acting on caspase-9, caspase-3 and Bim (13).

Also, studies on this subject were accomplished at the cell culture level, and most of the studies were realized by using rodents. In the literature, there have been a few studies demonstrating the role of miRNA-582-5p and miRNA-363 on caspase-9 activity in GBM. However, no studies have been found to date in serum or plasma samples of patients diagnosed with GBM.

In the scope of this work, it as aimed to determine roles of miRNA-582-5p and miRNA- 363 on caspase-9 levels by using the serum samples of GBM diagnosed patients and undiagnosed groups of this disease.

Also, caspase-9 gene Ex5+32 G>A (rs1052576) polymorphism was investigated in GBM patients and undiagnosed individuals in the Turkish population.

2. LITERATURE REVIEW

2.1. Overview of the Brain Tumors

Brain tumors were collected under two main groups as primary and secondary tumors (14).

Primary brain tumors are those that originate inside of brain tissue (15). They can be labeled by the type of tissue where they are sourced from. Glioma is a general term that describes all primary brain tumors. Phylogenic roots of the tissue hold a key role in determining the stages of primary malignant brain tumors. There are several types of gliomas. These include astrocytic tumors, which come off from astrocytes (astrocytoma, anaplastic astrocytoma and glioblastoma), oligodendrogliomas that rise in the cells which produce myelin and ependymomas which mostly evolve in the lining of the ventricles (Figure 2.1-1) (16-19). Statistical analyses showed that they are responsible for almost 80% of all malignant primary tumors of the brain (18-20).

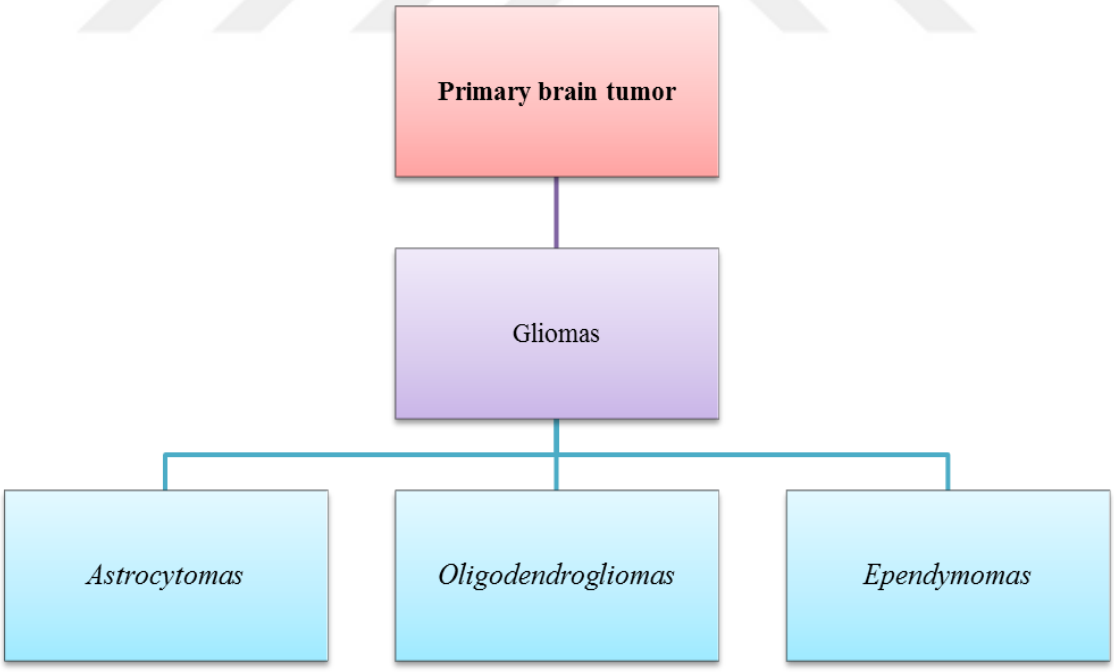


Figure 2.1-1. Classification of primary brain tumors and subtypes of glioma (16, 17).

Tumors caused by cancer cells originating from another part of the body that effuse to the brain (metastasis) are called secondary brain tumors. These types of tumors do not share the same features with primary brain tumors (21). Treatment for secondary brain tumors bound up with the origin of the starting point of cancer cells. Also, the age of patients and general health status are other important factors (22).

2.1.1. Glioblastoma Multiforme

Glioblastoma multiforme is the most widespread malignant type of tumor, and constitutes the majority of astrocytic tumors. When astrocytic tumors are classified according to the international grading system of the World Health Organization (WHO), tumors can fall under four classes identified by histopathological hallmarks (23). They can be classified from Grade I, which represents the most optimistic features to Grade IV, which holds the most deadly characteristics. GBM has been defined as Grade IV by WHO (23, 24) (Table 2.1.1-1). When the new grading system of 2016 central nervous system (CNS) WHO is analyzed, it is clearly seen that the molecular point of view (IDH-1/2 status etc.) has a strong influence on new classifications of CNS tumors, as compared with the 2007 CNS WHO report (24).

Also, it is liable for approximately 70% of all brain tumors in adults (25). It has been reported that each year 3 in 100,000 people are diagnosed with this destructive disease. The average lifetime of patients diagnosed with this disease is 14 months following diagnosis (26, 27).

Table 2.1.1-1. WHO classification of astrocytic tumors and their characteristic features (22, 23).

WHO Grade	CNS Tumors	Histological features	Age at diagnosis
I	Pilocytic astrocytoma	Microcysts, Rosenthal fibers	10
II	Diffuse astrocytoma	Mildly increased cell number or atypia	34
III	Anaplastic astrocytoma	Mitoses, prominent atypia	41
IV	Glioblastoma Multiforme	Necrosis, endothelial proliferation	53

Some of the major molecular and histological features of GBM include a high growth rate, resistance against apoptotic factors, tissue invasion, angiogenesis, vascular proliferation and necrosis (28). Also, one of the important characteristics of GBM can be understood from the name of the disease. The word "multiforme" provides information regarding tumor heterogeneity. Tumor heterogeneity has influence upon morphologies, growth rate, drug responses and gene expression levels of tumor cells (29, 30).

Two GBM subtypes have been identified, namely primary and secondary GBMs (31). Primary GBM is mostly diagnosed in elderly patients (31). It is seen that this type of GBM exhibits an aggressive tumor feature with high invasion, and usually there is no pre-clinical evidence for its presence (31). On the other hand, there are some different clinical progressions in secondary GBM; the most important difference is that it is seen in young individuals. Young patients with low-level glioma can be diagnosed with glioblastoma within 5 years after first diagnosis (31) (Figure 2.1.1-1).

It is known that 95% of GBM brain tumors are located in the cerebral hemisphere (32). Imaging techniques, such as catheter angiography, which is an invasive method, and computed tomography (CT) and magnetic resonance imaging (MRI), which are non-invasive procedures (33), showed that such kind of tumors can also be located outside of the cerebral hemisphere; some of these regions include the brainstem, cerebellum and spinal cord (32).

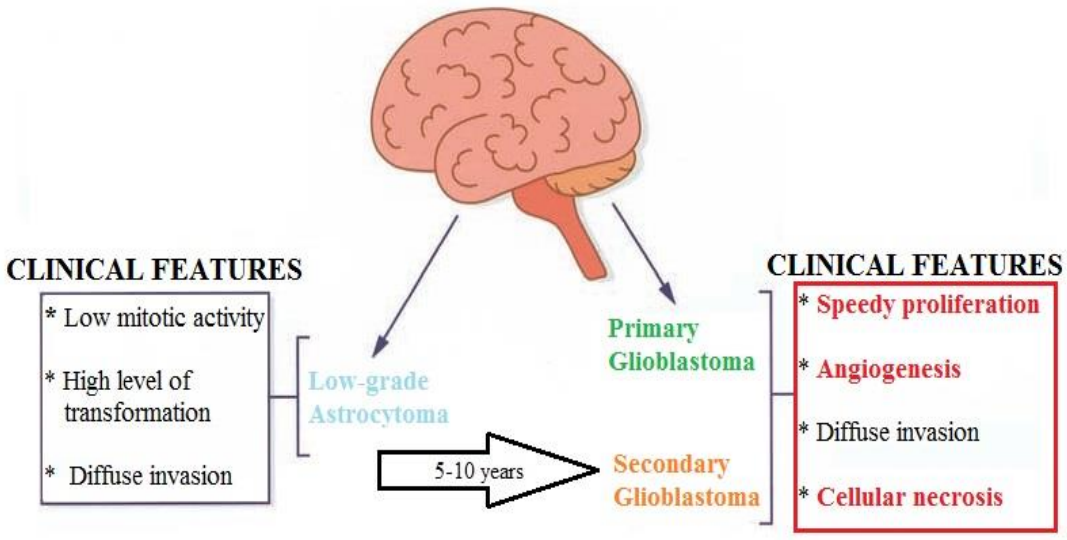


Figure 2.1.1-1. Two types GBM. Primary GBM can be the initial pathology at diagnosis. Secondary GBM can be rise from a low-grade astrocytoma over 5-10 years. Although they occur through different pathways share some clinical features. Adapted from reference (17).

In addition, there are also some molecular characteristic differences between the two subgroups. When the molecular groundworks of the subgroups of glioblastoma are examined, it is seen that while hallmarks of primary GBM are epidermal growth factor receptor (EGFR) gene mutation and amplification, over-expression of mouse double minute 2 (MDM2), deletion of p16 and loss of heterozygosity (LOH) of chromosome 10q holding phosphatase and tensin homolog (PTEN) and telomerase reverse transcriptase (TERT) promoter mutation, the characteristic features of secondary GBMs are over expression of platelet-derived growth factor A, platelet-derived growth factor receptor alpha (PDGFA/ PDGFRa), retinoblastoma (RB), LOH of 19q and mutations of isocitrate dehydrogenase 1 and 2 (IDH1/2),TP53 and ATRX (19, 34-37).

Despite the new therapeutic approaches against GBM, it remains as still a deadly disease with insufficient prognosis. The lead of standard treatment is surgical methods (38). However, GBM is not a type of tumor that can be treated completely with surgical procedure (39). Therefore, treatment applications are performed by combined methods such as radiation therapy and chemotherapy (40).

Some chemotherapeutic agents which are used for the treatment of brain tumors are namely Carmustine (BCNU), Lomustine (CCNU), Temozolomide (TMZ) and Cisplatin (41). In the treatment of GBM, TMZ which is a methylating agent, BCNU and CCNU have shown positive results (39). On the other hand for GBM patients' treatments, TMZ is the only standard chemotherapeutic agent (42).

As previously mentioned, the combined treatments that support each other have become standard treatments of GBM patients. Oral administrations of TMS and concomitant radiotherapy have become the standard care in practice for GBM patients (39). TMZ, which is a small alkylating agent, performs its function by methylating the DNA molecule at two points located on Guanine. As a result of this methylation process the cell cycle is stopped and apoptosis is triggered (43).

Although TMZ has the lowest toxic effect when compared to other agents, it has many side effects and cannot serve as an adequate agent due to the resistance by the tumor cells (44, 45). Thus, the lack of sufficiently responsive treatment methods leads to continuation of the new levels of treatment strategies for GBM patients, specifically at the molecular level.

2.2. Apoptosis

Apoptosis is the main biological process of programmed cell death that takes on a very crucial task in biologically indispensable processes such as embryonic development and homeostatic mechanisms of tissues (46, 47).

Cell shrinkage, chromatin condensation, inflated mitochondria and disrupted cytoplasmic membrane are some of the morphological changes occur in cells that undergo apoptosis (47).

One of the notable hallmarks of cancers, such as GBMs, is apoptotic resistance (48).

In mammalian cells, there are two well-identified apoptotic pathways the intrinsic and extrinsic pathways (Figure 2.2-1).

The extrinsic pathway is also called the death receptor pathway. This pathway can be activated apart from the cell. Key triggering molecules are pro-apoptotic ligands that have integration with death receptors (DRs). Pro-apoptotic ligands belong to the tumor necrosis factor (TNF) superfamily, such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and CD 95 (APO1/Fas). These ligands bind to the death receptors (TNF-R, FAS, DR3, DR4, DR5, and DR6) and, cause aggregation of the receptors (49). As result of the aggregation, Fas-associated death domain (FADD) and caspase-8 come together. Resulting from interactions, the death inducing signaling complex (DISC), which includes DR, FADD and caspase-8 come about (47).

Stress signals commence the mitochondrial pathway by apoptotic factors such as cytochrome c, apoptosis inducing factor (AIF) and Smac (second mitochondria-derived activator of caspase)/DIABLO (direct inhibitor of apoptosis protein (IAP)-binding protein with low PI), all of which locate at mitochondrial intermembrane spaces (47). Caspase-3 activation is provided by apoptosome complex, whereas caspase activation is promoted by Smac/DIABLO via blockage of the inhibitory effect of the IAPs (47).

The intrinsic pathway/mitochondrial pathway is triggered from inside the cells and controlled by Bcl-2 family proteins. Chemotherapy, radiation and cellular stress initiate the activation of the intrinsic pathway (50).

These factors lead to DNA damage by which the tumor suppressor p53 becomes activated. Puma and Noxa belong to the proapoptotic Bcl-2 family. Expressions of these Bcl-2 family members are regulated by p53, which is a tumor suppressor. In this way, activation of Bcl-2-associated X protein (Bax) /Bcl-2 antagonist/killer-1 (Bak) becomes simple, and Cytochrome C (Cyt C) is released into the cytosol (51).

In the cytosol, three key factors come together (Cyt C, apoptotic protease activating factor 1 (Apaf-1) and procaspase-9). This important group forms a complex called the apoptosome. This unique combination activates caspase-9 to its active form (52). Activation of caspase-9 enables the effector caspases, -3, -6, -7, thus resulting in apoptosis. At the end of this trigger series apoptosis is executed (53).

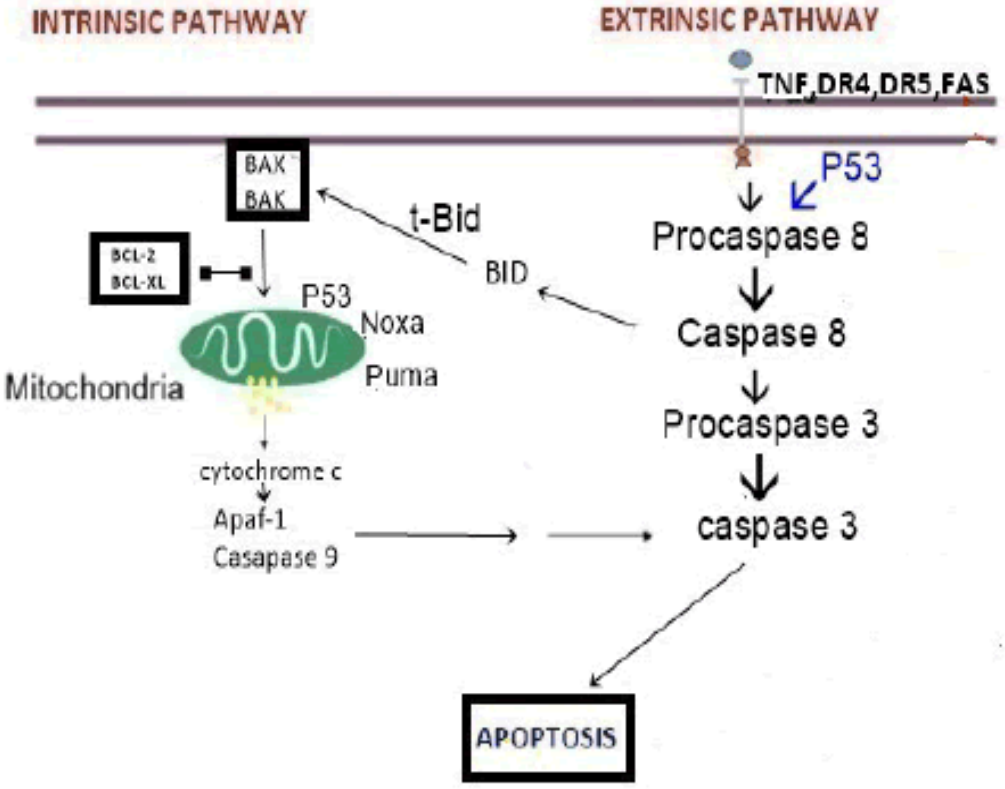


Figure 2.2-1. Intrinsic and extrinsic apoptotic pathways (54).

Research has shown that there is a link between these two apoptotic pathways at different levels. For example caspase-9, which has become active, causes the Bcl-2 family protein Bid turns back to the mitochondria and Cyt C can be released to trigger the mitochondrial amplification loop (55). The induction of apoptosis is significant for the development of cancer treatments (56). Therefore, researches originating from this approach continue to increase.

2.2.1. Caspases

Taking into consideration all of the complex pathways mentioned above, the key role of caspases is realized.

In mammals, the caspase family members, which include cysteine aspartic proteases, hold fundamental regulatory roles during the apoptotic process (12).
They cleave peptide bonds which are located following aspartic acid. More than a hundred targets are cleaved via 3 main kinds of caspases (57).

According to their functions, caspases are divided into 3 main groups:

I- Initiator Caspases (Caspase-2, -8, -9, -10)
II- Effector Caspases (Caspase-3, -6, -7)
III- Inflammatory Caspases (Caspase-1, -4, -5, -11, -12, -13, -14)

Caspase-2, -8, -9 and -10 are considered to be initiator caspases, whereas caspase-3, and to a lesser extent caspase-6 and -7, serve as effector caspases (58).
Caspase-9 is regarded as the canonical caspase in the intrinsic mitochondrial pathway (59).
Diverse cellular stresses trigger caspase activation by promoting the release of mitochondrial components, including Cyt C into the cytoplasm.
In turn, Cyt C promotes the assembly of a caspase-activating complex termed the apoptosome (60).

2.2.2. Caspase-9

The caspase-9 gene is located in the 1q36.21 region of the human chromosome 1 (Figure 2.2.2-1), which has 11 transcripts, 141 orthologues, and 17 paralogues (61). The caspase-9 gene can also be referenced by these abbreviations; APAF-3, ICE-LAP6, MCH6, PPP1R56 (61).

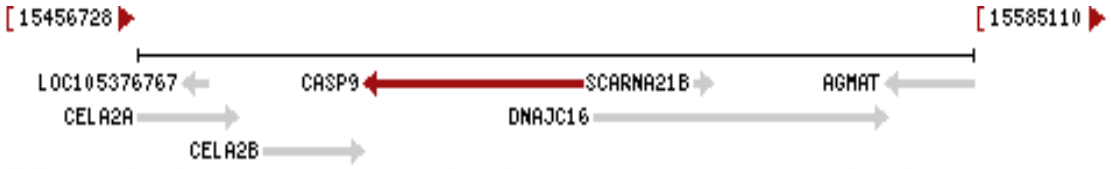


Figure 2.2.2-1. Human Chromosome 1 (61).

In the beginning, caspase-9 is synthesized as a single strand inactive form called a zymogen, as is the case with all caspase family members. Procaspase-9 is the inactive form of caspase-9, and it is a precursor caspase of the mitochondrial apoptotic pathway (12).

Caspase-9 is cysteine aspartic protease, which has an irreplaceable role in the mitochondrial death pathway (the intrinsic pathway). *the Bcl-2 family* and *the BH-3 (Bcl-2 homology domain-3)-only* proteins have took over important roles in regulation of caspase-9 (58, 59).

It contains a special motif CARD (caspase activation domain), which takes place at the Apaf-1 structure (Figure 2.2.2-2). This motif supplies selectivity to the binding process of caspase-9 (62).

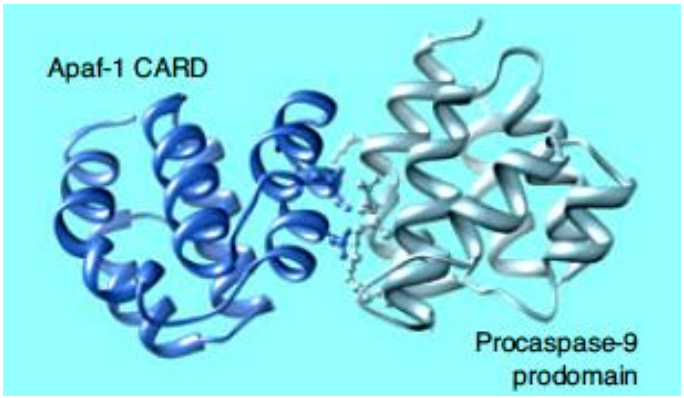


Figure 2.2.2-2. Molecular structure of Apaf-1 and CARD motif with Procaspase-9 (50).

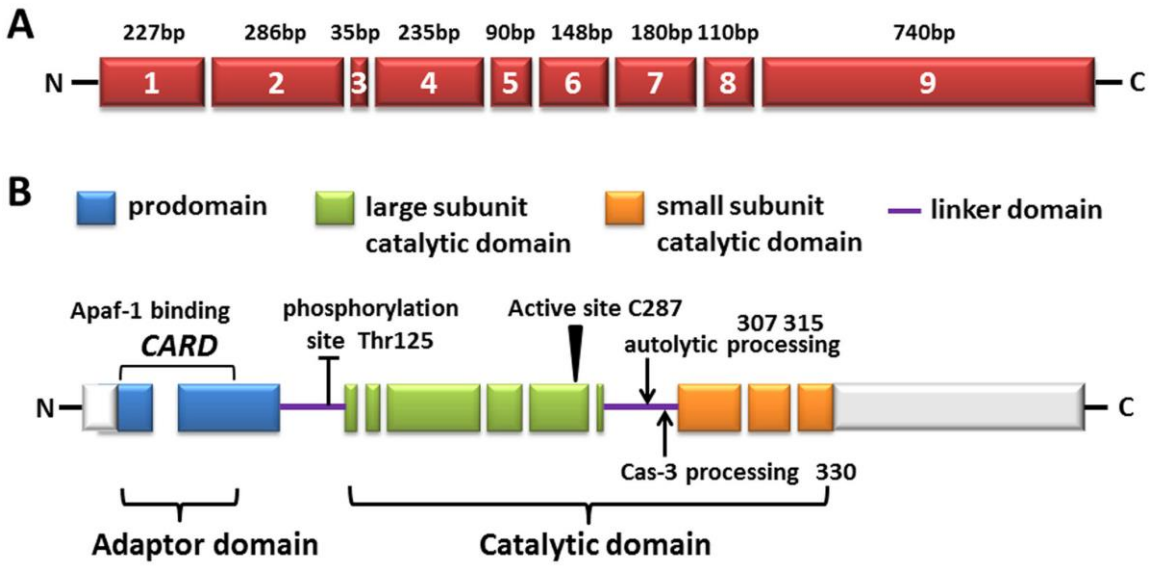


Figure 2.2.2-3. The Human Caspase-9 a) Exons and their lenght of Caspase-9 b) Amino acid structure and domains of caspase-9 (63).

The dimerization process has an initiator effect on caspase-9 activity. This process triggers autocatalytic cleavage, which results in the production of the active form of caspase-9 (64, 65).

Two hypothesis models are identified for caspase-9 activation. These are the "induced conformation model" and the "induced proximity model". Under the "induced conformation model", the apoptosome complex binds to caspase-9 for conformational changes (66, 67).

Hu and colleagues have supported this activation model by their work on subject (68). They have showed that caspase-9 and CARD domain of Apaf-1 have a multimeric interaction (68). Another hypothesis assumes that apoptosome complex formation provides a basis for caspase-9 dimerization (66, 67).

Caspase-9 can be regulated by multiple internal and external factors. This complex situation has encouraged the scientific world to accomplish even more detailed investigations about caspase-9 and its regulators. Nowadays, it has been reported that there are many regulatory candidates like miRNAs.

Caspase-9 can be regulated by some endogenous regulators that show effects of either inhibition or enhancement. While ERK2, Akt/PKB, HAX-1 and NO show their effects by inhibiting the caspase-9 activity, c-Abl, Nucling and NAC/DEFCAP show their effects via enhancement of caspase-9 activity (63).

Recent research has revealed the presence of tissue-specific miRNA expression. As in other cancer types, disease-specific miRNA expression models are also seen in GBM (69-72).

Cancer researchers have identified some miRNAs which have an effect on cancer development like miR17, miR-34 and miR-145 (73-74).

Several microRNAs, such as miRNA-124, miRNA-7, miRNA-128, and especially miRNA-21, have been reported to be highly expressed in GBM compared to normal brain tissue (73-74). These miRNAs have also been reported to have a role in neuronal differentiation (75).

Also, recent studies have shown that miRNAs can play a role in the regulation of caspase-9 activity.

Only a few of miRNAs are shown to be regulators of caspase-9 (Table 2.2.2-1).

Table 2.2.2-1. Caspase-9 and its regulatory miRNAs (63).

miRNA Type	**Target**
miRNA-23a	Caspase-9
miRNA-24a	Apaf-1 and caspase-9
miRNA-582-5p	Caspase-9

So far, Floyd and colleagues have completed a important study on the relationship between caspase-9 and miRNA in GBM (13). miRNA-582-5p and miRNA-363 have been selected as target miRNAs in GBM cell lines by using the Microarray method. They have put knowledge about expression levels of miRNA-582-5p and miRNA-363 and their effects on apoptotic activity. These selected miRNAs show their effects by inhibiting the expression levels of caspase-3 and caspase-9 (Figure 2.2.2-4). By this way, the apoptotic process of the cell is blocked and tumor survival is maintained (13).

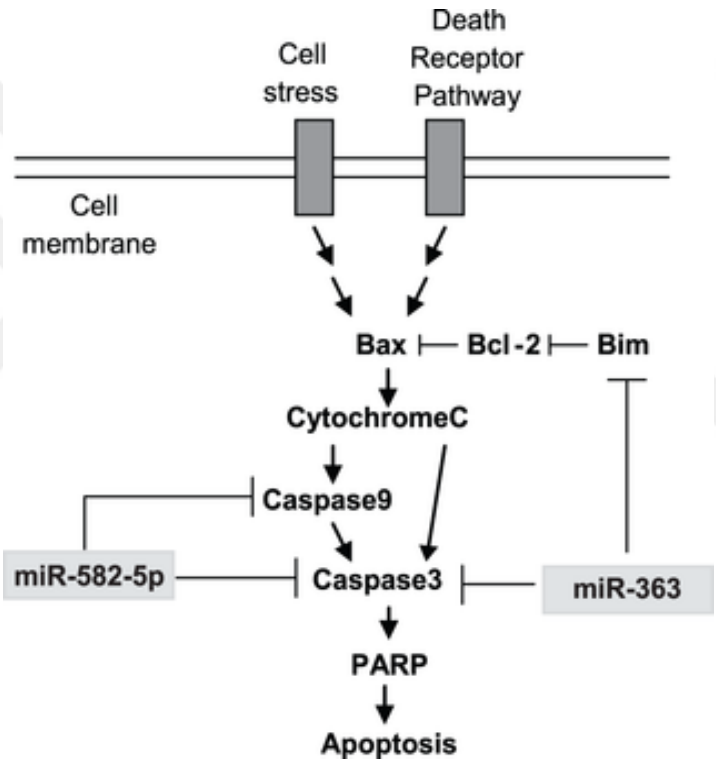


Figure 2.2.2-4. The apoptotic cascade (13).
A graphical representation of the miRNAs 582-5p and 363 targeting and decreasing expression of multiple apoptotic pathway components.

2.3. microRNAs

MicroRNAs (miRNAs) are endogenously encoded single-stranded RNAs that belong to the non-coding RNA family. They have negative regulatory effects on gene expression (76).

In 1993, the first miRNA was identified by Victor Ambrose et al. in *Caenorhabditis elegans*, and it was referred to as *lin-4* (77).

Until today, extensive researches have been accomplished, and they have demonstrated the importance of miRNAs in several important biological processes (78, 79).

In all genome studies performed in recent years, it has been demonstrated that the human genome transcribes thousands of noncoding RNAs. Some of those noncoding RNAs are microRNAs (miRNA), piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNA) and long noncoding RNA (lncRNA) (80) (Figure 2.3-1).

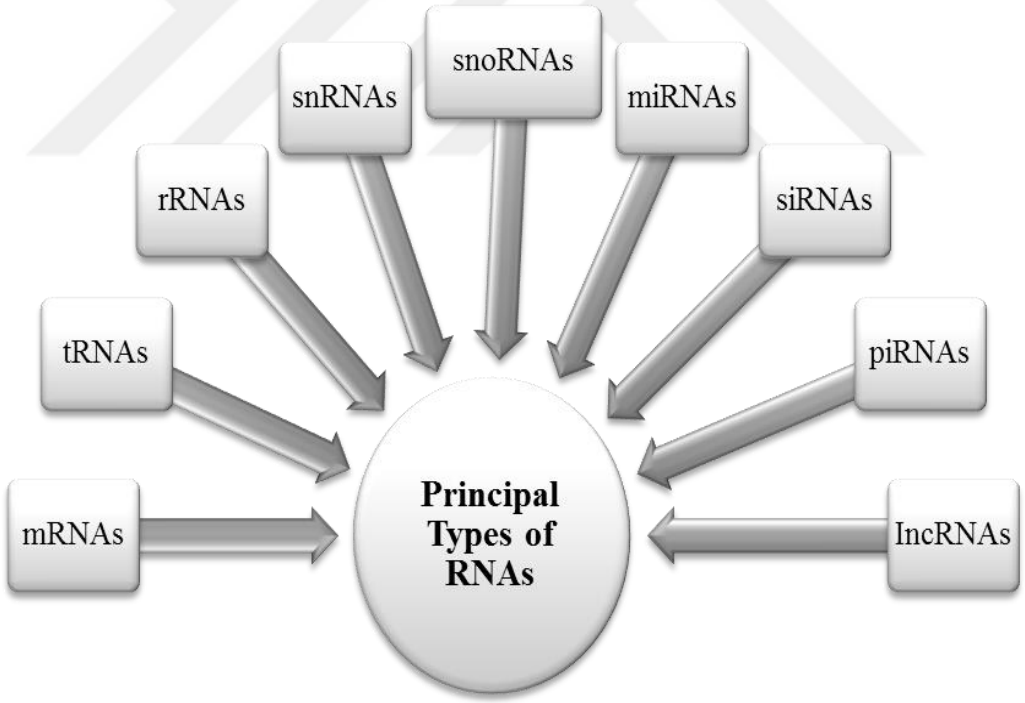


Figure 2.3-1. Principal Types of RNAs Produced in Cells (91).

2.3.1 microRNA Biogenesis

The biogenesis of miRNAs has been grouped into canonical and non-canonical pathways (81).

Research, that has focused on the human genome have demonstrated that the human genome has protein coding (exons) and non-protein coding regions (introns). miRNAs are named according to the genomic domain to which they are proceeded, and they can be described as intergenic or intragenic miRNAs (82). The majority of miRNAs, which are transcribed from introns that do not encode any protein have been called intragenic miRNAs. The other miRNAs are called intergenic miRNA which are transcribed independently from host genes (83, 84).

The vast majority of miRNAs are processed by using the canonical pathway. This process occurs in the nucleus by the activity of the RNA polymerase II molecule (85). Primer-miRNAs (pri-miRNAs) are synthesized from mRNAs in the nucleus. pri-miRNAs contain approximately 70 nt., and they have a hairpin structure created by themselves (86). Another key enzyme of the process is RNase III which belongs to the endonuclease family. It has serine-proline-arginine rich regions in its N-terminus.

This enzyme comes together with Drosha, which is also known as the microprocessor, and DiGeorge Syndrome Critical Region-8 (DGCR8) to obtain a complex structure (87). pri-miRNAs can be cleaved by Drosha by help of DGCR8. DGCR8 has a unique ability to recognize special motifs such as N6-methyladenylated GGAC motif is that inside of the pri-miRNA (88). Following a cutting procedure precursor miRNA (pre-miRNA) is generated. One of the obtained pre-miRNA strands is transported to the cytoplasm with the support of exportin 5 (XPO5), which is a nuclear transport receptor combined with RanGTP. Dicer is a member of the ribonuclease III (RNase III) endonuclease enzyme family located in the cytoplasm. pre-miRNAs are cut into the cytoplasm by Dicer enzyme, and two approximately 23 nt. long mature miRNAs are formed (89). When one of the obtained pre-miRNA strands called the guide strand is loaded into the RISC (89).

Structural analyses have shown that RISC includes Dicer enzyme, Argonaute 2 (AGO 2) protein and Transactivating Response RNA Binding Protein (TRBP); another strand is called the passenger strand and it is degraded. Researchers and their working groups have identified a molecule called GW182 (90). This molecule takes place at the RISC structure and helps to stabilize AGO2 (90).

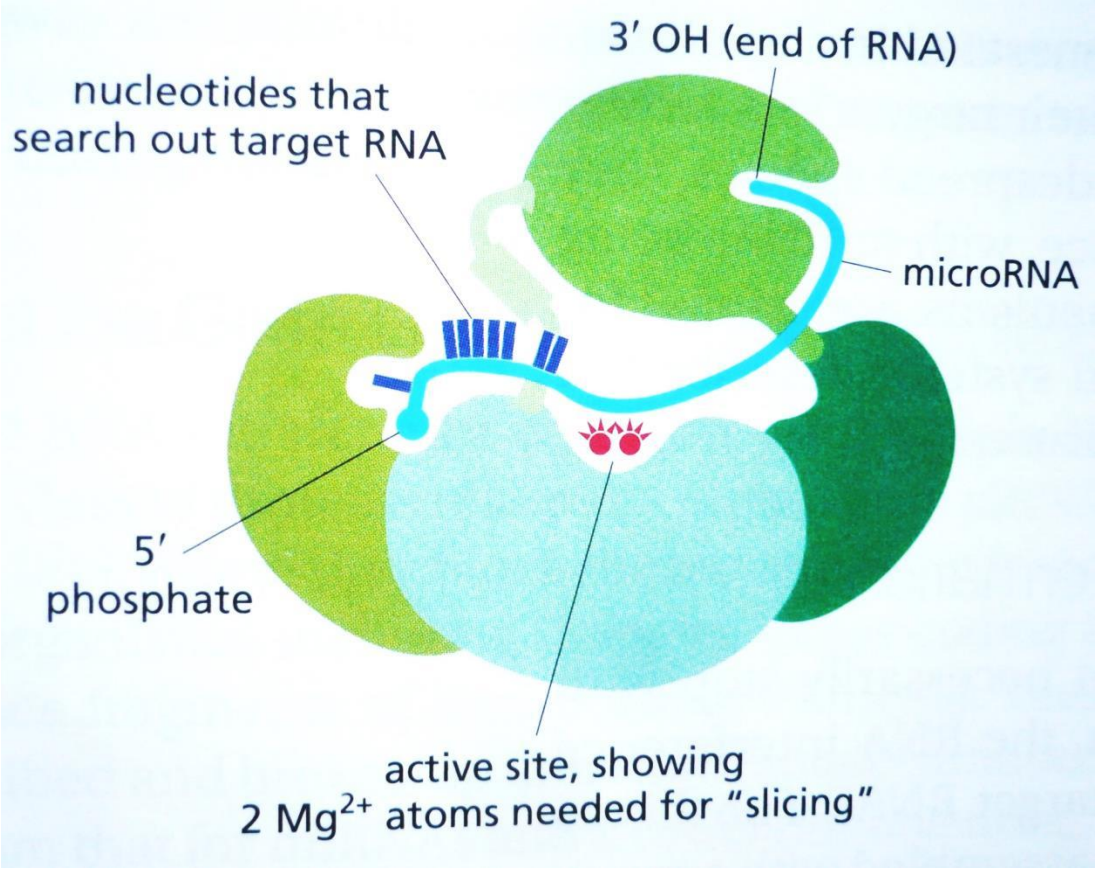


Figure 2.3.1-1. Human Argonaute (AGO) protein with target miRNA (91).

mRNA degradation or repression of mRNA synthesis is triggered through guide strand and RISC interaction. In conclusion, target mRNA expression profiles are organized posttranscriptionally (92-95)

Studies have reported that miRNAs can bind to a specific sequence on 3′ Untranslated Region (UTR) and 5′UTR coding sequences and be promoter region of their target miRNAs (96). By the binding of miRNA to the 3′ UTR region causes the deadenylation, decapping and transcription of mRNA (97, 98).

miRNA-RISC (miRISC) complex binds to its special sequence of target mRNA. The seed region is a highly conserved sequence of the 5'-prime (-p) part of the target mRNA. Interaction of the miRISC complex and target mRNA is achieved through this 2 nt. length region (99).

mRNA degradation is achieved by a deadenylation process. CCR4-NOT and poli (A)-nucleases 2-.and 3- (PAN2 and PAN3) deadenylases form a complex, which degrades the 3'-p of the poly-A tail of the target mRNA by means of the GW182 protein (90).

Another way for mRNA degradation process can occur by degradation of 7-methylguanosine (m^7G). mRNA degradation is accomplished by the combined activity of DEAD –box helicase 6 (DDX6) and CCR-4-NOT complex with decapping the mRNA 2 (DCP2) (90). In final part of mRNA degradation, activity of 5'-3' exonuclease 1 (XRN1) eliminates the remaining part of mRNA (100).

When miRNAs bind to the 5′ UTR and coding regions, gene expression of mRNA are silenced (101, 102). On the other hand, interaction with the promoter region is caused to induce the transcription (103). However, more studies are required to understand the underlying molecular interactions between mRNA regions and miRNAs.

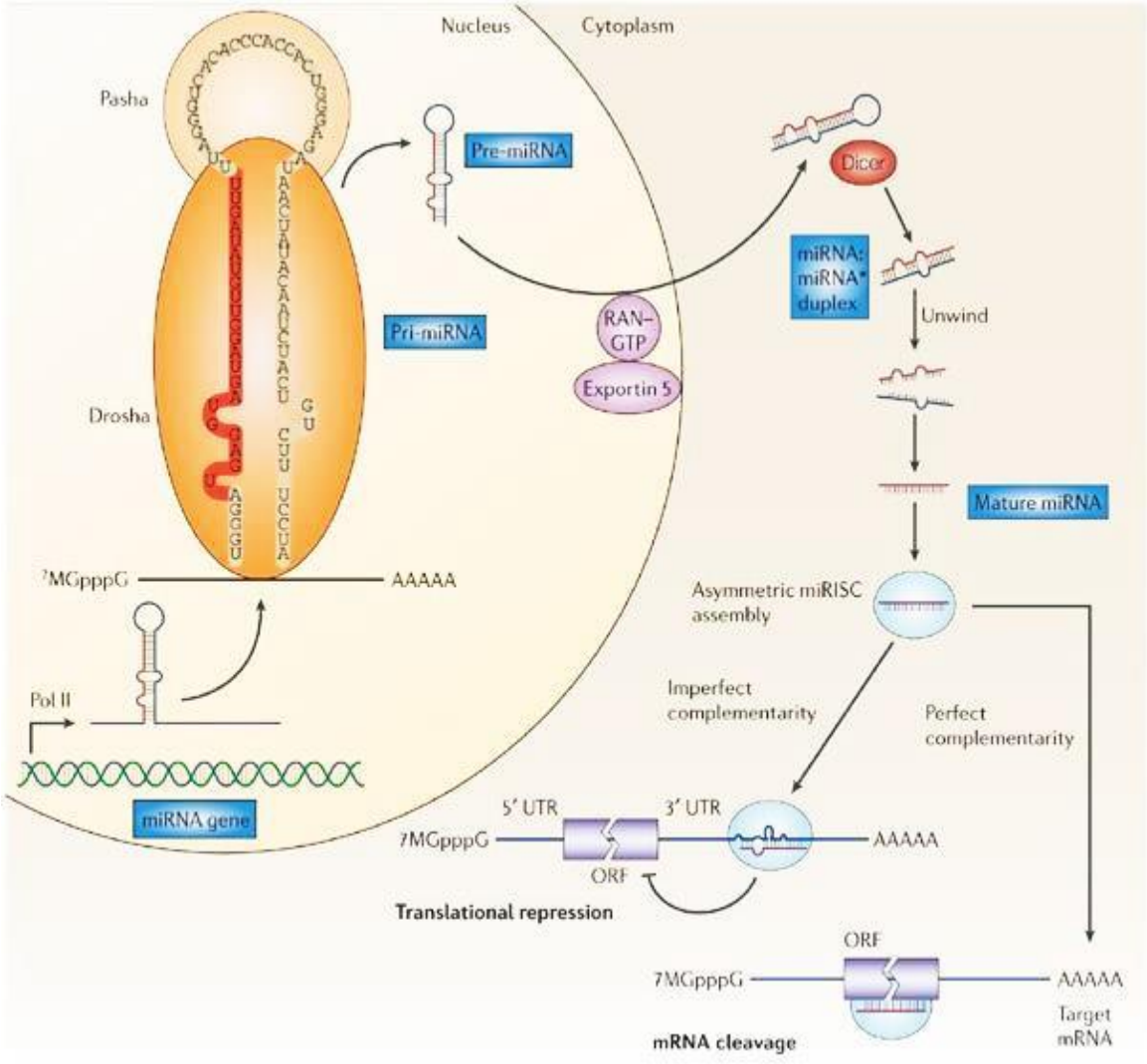


Figure 2.3.1-2. miRNA Biogenesis (104).

More than one non-canonical pathway was identified to the present (Figure 2.3.1-3).

As in the canonical pathway, molecules such as Drosha, Dicer and XPO-5 are involved in this pathway. Non-canonical pathways can be divided into two groups: Drosha/DGCR8 independent or Dicer-independent pathways (90).

In the Drosha/DGCR8-independent pathway a molecule named mirtrons is seen. This molecule is a pre-miRNA produced from introns of target mRNAs (105, 106).

Another different pre-miRNA molecule seen during the non-canonical pathway is m^7G capped pre-miRNA. This kind of pre-miRNA can pass into the cytoplasm by using exportin-1 without any need for cleavage by Drosha (107).

In the Dicer-independent pathway, Drosha molecule is used for the cleavage of short hairpin RNA (shRNA); by this way, miRNAs can be obtained (108).

All pre-miRNAs mentioned above require AGO2 protein for their maturation process.

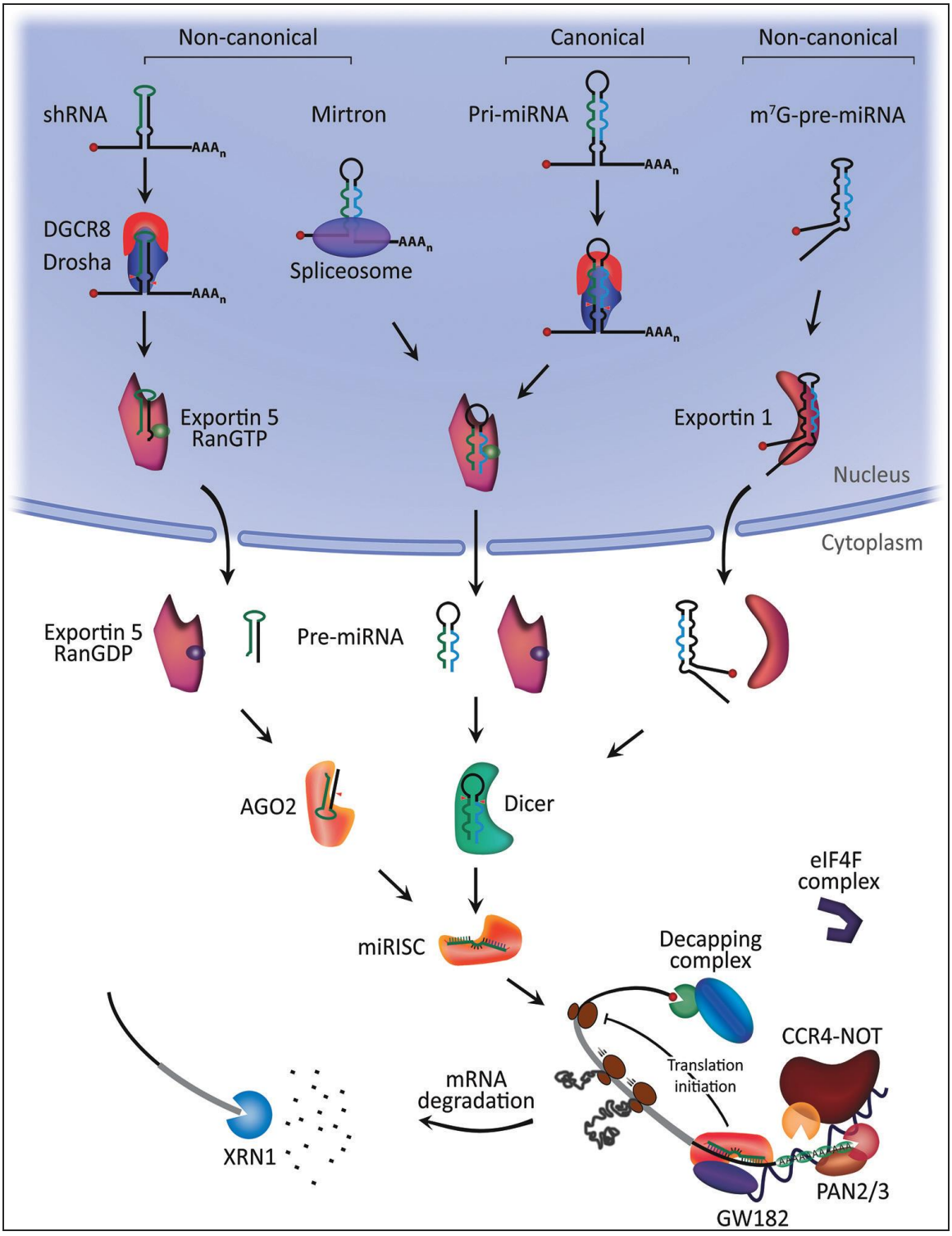


Figure 2.3.1-3. Canonical and Non-canonical pathways of miRNA Biogenesis (90).

2.3.2. microRNAs as Biomarker Candidates

Scientific studies have demonstrated that miRNAs can be released into extracellular fluids. These kinds of miRNAs can be used as biomarkers for several diseases (109-111).
Extracellular miRNAs carry by means of exosomes, macrovesicles, apoptotic bodies and proteins such as AGO2 (112, 113, 115).

Molecular diagnostic signs made from biologic ingredients are called biomarkers. In this context, miRNAs have become novel biomarker candidates especially in human cancer diagnosis (79, 116).
The main theme of recent studies, which focus on the relationship between miRNA and disease, are to determine whether miRNA is a disease-specific biomarker or not.

Some biological fluids of humans such as serum, plasma (115, 116), cerebrospinal fluid (117), saliva (106), breast milk (118), urine, tears, colostrum (119) and ovarian follicular fluid (120) give opportunity for diagnostic research (99).
The main goal of the scientific world in the future is to prevent diseases by using human samples to determine the targets of miRNAs and to develop techniques to increase or decrease miRNA expression levels (90).

There are some technologies which have been developed based on this purpose. miRNA-Mimic is a technology that attempts silencing of target genes (121). This technology has been developed as a solution for miRNAs that cannot be synthesized in sufficient amounts. This method shows its activity by synthetically synthesized miRNAs, which have been found not to be sufficiently synthesized.
Synthetically synthesized miRNAs can bind to the 3’ UTR regions of their target mRNAs. However, it is known that a miRNA can target more than one mRNA. Therefore, it can be said that it is not a reliable method (121).

On the other hand, there are also diseases caused by the excessive expression of miRNAs. Based on this information, some methods have been developed aimed to reduce the expression levels of target miRNAs.

One of these kinds of methods is called the anti-miRNA technique, which has been developed for diseases caused by over-expression of miRNAs. In this technique, antisense oligonucleotides (ONs) are synthesized (122). These oligonucleotides degrade the miRNAs with the help of an unspecified mechanism.

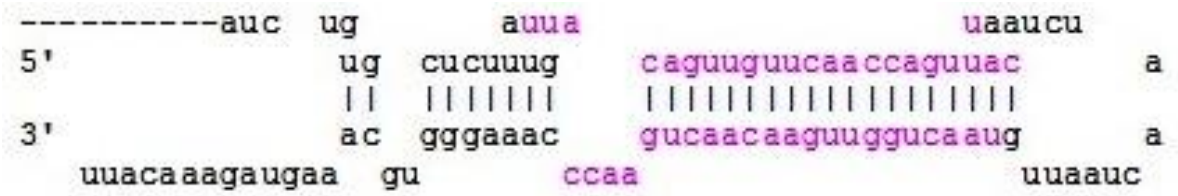
Another technique developed by Ørom and a working team is called the locked nucleic acid (LNA) system (123). In this system, the oxsomethylene molecule holds the key feature in prevention of expression of target miRNAs. This inhibition is induced by the conformational alteration of the ribose sugar. The oxymethylene binds to the 2nd and 4th carbons of the ribose sugar, and anti-miRNAs are mediated by increasing their susceptibility to the miRNAs they aim to prevent (124).

The two microRNAs, which are the subject of this thesis (miRNA-582-5p and miRNA-363), have potential to serve as new biomarker candidates for GBM (13).

2.3.3 miRNA-582-5p

miRNA-582-5p is a member of the microRNA family including 23 nucleotides (125). There is no information about whether this miRNA belongs to any miRNA cluster or not.

(a)



(b)

Figure 2.3.3-1. Mature miRNA-582-5p
a) pre-miRNA-582-5p which is the precursor of mature miRNA-582-5p
b) Structure of mature miRNA-582-5p (126).

q12.1 location of the human chromosome 5 plays host to the MIR-582 gene (Figure 2.3.3-2). MIR-582 gene has 1 transcript and 67 orthologues (126).

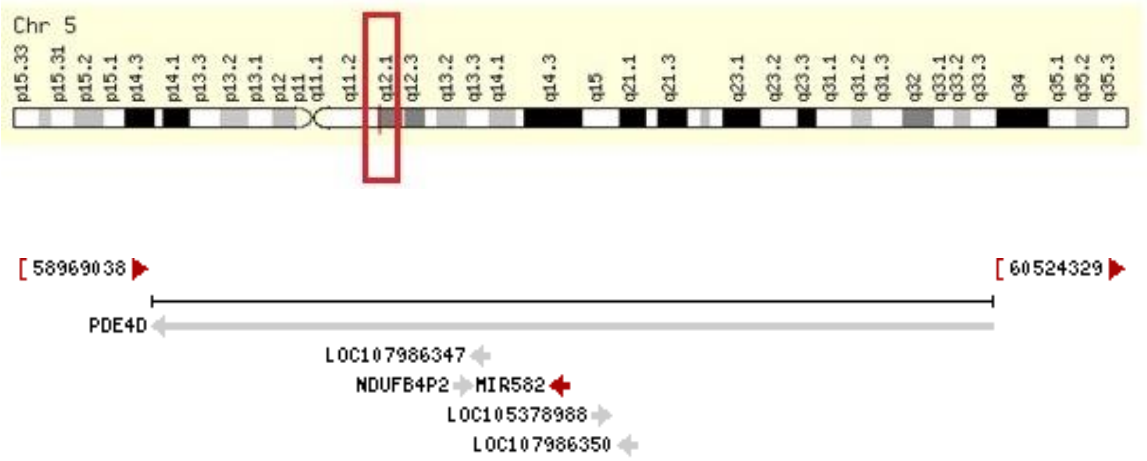


Figure 2.3.3-2. The region where miRNA-582-5p is located on the 5th chromosome MIR582 Gene in genomic location (127).

miRNA-582-5p has been shown to be associated with several biological progressions of cancer cells.

In 2013 Uchino et al., reported that miRNA-582-5p has an inhibitory effect on tumor proliferation in bladder cancer. They also showed that miRNA-582-5p and -3p have three mRNA targets, which are PGGT1B, LRRK2, and DIXDC1. This miRNA affects the progression of highly malignant bladder cancers (128).

According to another study performed in 2013, -5 and -3 p of miRNA-17 have an effect on tumor growth by targeting TIMP3 (129).

Zhang and his team in 2015 (130) performed a study with miRNA-582-5p as it relates to human colorectal carcinoma. They put forth that overexpression of miRNA-582-5p in human colorectal carcinoma inhibited cell proliferation, cell cycle progression and invasion.

According to a published article in 2017, phosphatase and tensin homolog were targeted to affect tumor formation (131).

Although there are a lot of newly published works on the topic, its biological role and underlying molecular mechanism are still not clear in the case of cancer.

2.3.4 miRNA-363

miRNA-363 is a member of the miRNA 25/29 family (Table 2.3.4-1) (obtained from 22 nucleotide) (132).

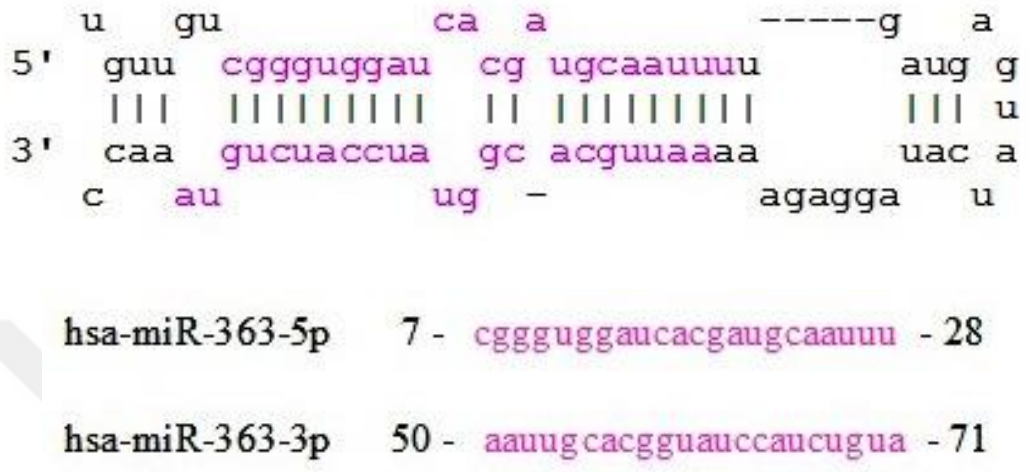


Figure 2.3.4-1. Mature miRNA-363
a) pre-miRNA-363 which is the precursor of mature miRNA-363
b) Structure of hsa-miR363 (133).

miRNA-363 gene takes place on the Xq26.2 region of Chromosome X (Figure 2.3.4-2). This gene has 1 transcript and 81 orthologues (132, 133).

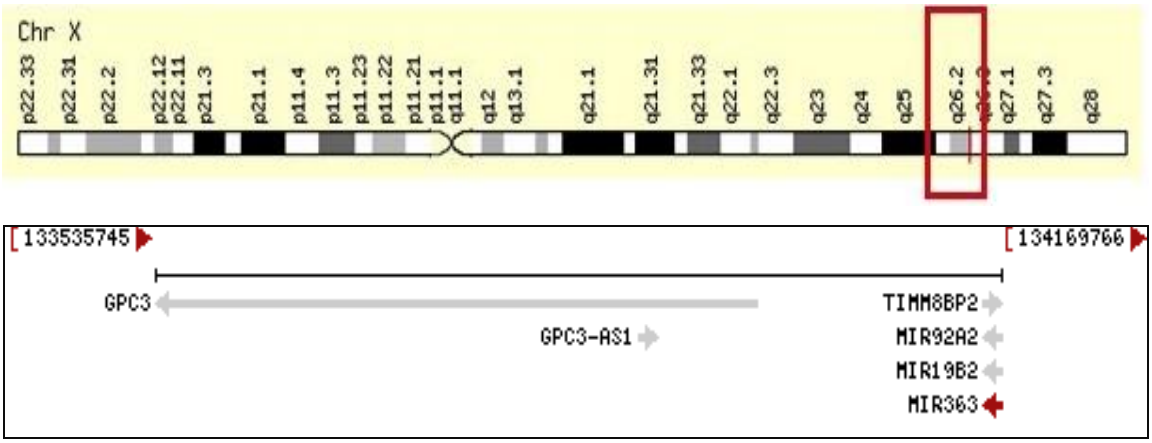


Figure 2.3.4-2. MIR582 Gene in genomic location on the chromosome 5 (134).

The role of miRNA- 363 in many diseases has been shown through previous articles, as is the case with other described micro-RNAs.

Table 2.3.4-1. Member of the miRNA 25/29 Family (135).

NAME	APPROVED SYMBOL	SYNONYMS	CHROMOSOME
microRNA 25	MIR25	hsa-mir-25	7q22.1
microRNA 92a-1	MIR92A1	hsa-mir-92a-1	13q31.3
microRNA 92a-2	MIR92A2	hsa-mir-92a-2	Xq26.2
microRNA 92b	MIR92B	hsa-mir-92b	1q22
microRNA 363	**MIR363**	**hsa-mir-363**	**Xq26.2**

Takahashi et al reported that miRNA-363 is overexpressed in CD4 (+) and CD8 (+) CB cells in human cord blood and in adult peripheral blood cells upon proinflammatory stimulation (136), which suggests its immunomodulatory role in inflammatory diseases including RDS.

3. MATERIALS AND METHODS

3.1. The Patient Population and The Study Protocol

Whole blood and serum samples of the patient group (n= 35) and control group (n=36) were obtained from the Yeditepe University Hospital Neurosurgery Clinic within the scope of the Clinical Research Ethics Committee with corresponding decree no: 916.

Working criteria are;

- Age range of 18-70 years
- Glioblastoma multiforme diagnosis to be included in the patient group
- No Glioblastoma multiforme diagnosis for the control group

In this work, samples were chosen according to the patients who had gone to the neurology polyclinic with complaints of a headache, vomiting, faint. Physical examination, neurological examination, optic examination and radiological examination detected disease symptoms.

Cytosolic $NADP^+$ related isocitrate dehydrogenase (IDH-1) status of the GBM patient group was examined by usig immunohistochemical methods.

3.2. Materials and Devices:

3.2.1. Preparation and The Separators:

3.2.1.1. DNA Isolation

The DNA Isolation system mixture, which has a pH 8.8, contains 10.5mM Tris-Cl, 10.5mM NaCl, 10.5nM EDTA, 8M Guanidiniumhydrochloride and 1.12mg/ml Proteinase K (iPrep Purelink, Invitrogen, Thermo Fisher Scientific Inc).

3.2.1.2. microRNA Isolation

For the miRNA isolation procedure we utilized Qiagen's miRNeasy Serum/Plasma Kit. The experimental protocol involved lysis, homogenization, 70% Ethanol addition, washing and elution steps.

miRNA Isolation Kit (miRNeasy Serum/Plasma Kit, Qiagen) was used for isolation, and involved Trizol (Quiazol, Qiagen), Chloroform (Sigma Aldrich), 100% Ethanol (Sigma Aldrich), miRasy Spike in control (miRNA39, Qiagen) and RWT Buffer containing Guanidine salt.

RWT Buffer was prepared by adding 44 microliters (µl) ethanol immediately prior to use. RPE Buffer is used to eliminate salts and deposits handled in colons. 30µl ethanol was used for the preparation of the RPE Buffer.

Complementary DNA (cDNA) reverse transcription kit (miScript II Kit, Qiagen) involved RNase free water, miScript HiFlex Buffer, Nucleic Acid Mix and miScript Reverse Transcription Mix.

3.2.1.3. Determination of microRNA Levels by Fluorometer

Experimental solution was prepared by using miRNA specific reagent and miRNA buffer. Calibration of the fluorometer was done by using standard 1 containing 10 ng/µl and standard 2 containing 250 pg/µl rRNA. During measurement, working solution and RNA were put into the 500 µl Qubit tubes (Qubit 3.0 microRNA Assay Kit, Invitrogen, Thermo Fisher Scientific Inc.).

3.2.1.4. Purity Determination by NanoDrop

DNase-RNase free 18m ohm water was used.

3.2.1.5. Detection of microRNA Expression Levels by Real-Time Polymerase Chain Reaction (RT-PCR)

microRNA Universal Primer (Qiagen), miScript SYBR Green PCR Kit (Qiagen), microRNA Primer Assay (miRNA-582-5p and miRNA-363, Qiagen), microRNA Housekeeping Assay (RNU6, Qiagen), Strip Tubes and Caps 0.1 ml (Qiagen) and DNase-RNase free 18m ohm water were used.

3.2.1.6. Detection of Serum Caspase-9 Expression Levels by ELISA

Human caspase-9 Elisa Kit (Affymetrix, eBioscience). Monoclonal antibody coated microwell plate, anti-human caspase-9 polyclonal detection antibody (rabbit), anti-rabbit-IgG-HRP, human caspase-9 standard, sample diluent, assay buffer (PBS with 1% Tween 20, 10% BSA), wash buffer (PBS with 1% Tween 20), lysis buffer, substrate solution (tetramethyl-benzidine), stop solution (phosphoric acid) were used.

3.2.1.7. Detection of Caspase-9 Polymorphism by Real-Time Polymerase Chain Reaction (RT-PCR)

7500 Fast-Real Time PCR (Applied Biosystems), TaqMan Genotyping Assay, TaqMan Genotyping Master Mix, Real Time PCR 96 Well Plate (Thermo Fisher Scientific Inc) and DNase-RNase free 18m ohm water were used for detection of Caspase-9 polymorphism.

3.2.2. The Equipments:

DNA Isolation Robot (iPrep Purelink, Invitrogen, Thermo Fisher Scientific Inc), +4^{o}C Refrigerator (Haier), -20^{o}C Refrigerator (Haier), -80^{o}C Refrigerator (Haier), Vortex (V.I Plus Biosan), Automatic Pipette Kit (Thermo Fisher Scientific Inc.), Plate Centrifuge (Hettich), Centrifuge (Centrifuge 22R, Beckman Coulter), Ultra-pure water device (Purelab option Q, Elga), NanoDrop 2000 (Thermo Fisher Scientific Inc), Fluorometer (Qubit 3.0, Invitrogen, Thermo Fisher Scientific Inc), Real Time PCR (Fast Real-Time 7500, Applied Biosystems), Rotor Gene-Q Series (Qiagen), ELISA Plate Washer and an ELISA Reader.

3.3. Methods:

3.3.1. Genomic DNA Isolation from Blood:

Venous blood samples belonging to the patient and control groups were collected into 5cc tubes containing EDTA. These blood samples were stored at +4^{o}C until the start of the DNA isolation process. DNA isolation from the blood samples was realized using the iPrep DNA extraction robot and iPrep Genomic DNA isolation from blood Kit (iPrep gDNA Blood Kit).

For this experimental procedure, 350µl of blood was placed into the robotic system. The functioning of the system depends on the pH of the working area. Less than 7.0 pH positively charged ChargeSwitchTechnology® (CST®) binds to nucleic acid structure due to its negative charge. Positively charged proteins and other deposits do not bind to the bids. By utilizing washing steps, these proteins and deposits can be discarded from the working substance. In this technology, bids wash by means of elution buffer.

By this procedure, the surfaces of the bids are neutralized.

The entire process lasted 45 minutes, and 150 µl DNA were obtained at the end of the isolation steps. Obtained DNA samples for both groups were stored at +4^{o}C (137).

3.3.2. Purity Determination of Genomic DNA by NanoDrop

The purities of the isolated DNA samples were measured using NanoDrop 2000 instrument. Purity and concentration of the DNA molecules were determined by 260 nm and 280 nm wavelengths. OD260/ OD280 ratio between 1.7 and 1.9 was determined as the acceptable purity value. Genomic DNA concentrations were denoted in ng/µl units (138).

3.3.3. microRNA Isolation from Serum Samples

For the miRNA isolation procedure Qiagen's miRNeasy Serum/Plasma Kit was utilized. The experimental protocol involved lysis and homogenization, ethanol addition, washing and elution steps.

Blood samples of patients and healthy volunteers were collected into tubes. Serum samples were obtained by blood centrifugation at 4500 rpm for 15 min. Obtained serum samples were stored at -80ºC until use.

Prior to the miRNA isolation procedure, serum samples were incubated at 37 ºC to be melted.

200 µl of serum samples were put into the sterile ependorfs and 1000 µl lysis solution (Qiazol Lysis Solution) was added and stirred. After this process, samples were incubated at room temperature for 5 minutes and 3.5 µl "miReasy Spike in control" (1.6 x 108 copy/µl) were added to the mixture. 200 µl of chloroform were added to each sample under a fume hood and were incubated for 3 minutes then mixed for 5 minutes with a vortex instrument.

The experimental mixture was centrifuged at +4^{o}C at 12.000g for 15 minutes. After centrifugation, the experimental mixture had divided into two separate phase. The top phase of the mixture was transparent, and 600 µl of this transparent phase was taken into clean ependorfs. 900 µl of 100% ethanol was added on top, and then the mixture was vortexed. 700 µl from this mixture was passed through columns and centrifuged at 8000g for 15 seconds at room temperature. This process was repeated. 700 µl of RWT buffer was added on to the columns, and the supernatant was discarded after centrifugation at 8000g for 15 seconds. The same procedure was then repeated with 500 µl of RPE buffer. 500 µl of 80% ethanol was added into the same columns and centrifuged at 8000g for 2 minutes.

Following this process the supernatant was discarded. The columns were placed in new tubes and dryly centrifuged at a maximum speed for 5 minutes. Finally, 14 µl of RNA free water was added. The microRNA isolate was obtained following centrifugation at a maximum rate for 1 minute.

3.3.4. cDNA Synthesis

miRNA isolates, obtained through the previous stage were converted to cDNA using the reverse transcription process (miScript II Kit, Qiagen) (Table 3.3.4-1). These samples were stored at -20^{o}C until the date of the experiment (139).

Table 3.3.4-1. cDNA mixture for PCR Reaction

5x miScript HiFlex Buffer	4 µl
10x miScript Nucleic Mix	2 µl
miScript Reverse Transcriptase Mix	2 µl
RNA free water	10,5 µl
miRNA Sample	1,5 µl
Total volume:	**20 µl**

Table 3.3.4-2. Incubation Conditions for cDNA Synthesis

60 min	37°C
5 min	95°C
∞	95°C

3.3.5. Measurement of microRNA Purity

The purity of the miRNA samples were measured with the NanoDrop 2000 instrument. The purity of the miRNA samples was determined by the OD260/OD280 ratio. Samples accepted as pure held an OD260/OD280 ratio greater than 2 (140).

3.3.6. Determination of microRNA Levels by Fluorometer

The fluorometric method was used to determine the expression levels of the miRNAs. For this measurement process, 199 µl of miRNA Buffer and 1µl of miRNA Reagent (Qubit microRNA Assay, Invitrogen, Thermo Fisher Scientific Inc) were used, and the total volume was adjusted to 200 µl. For calibration of the device, two different standard solutions were prepared as standard 1 and standard 2. Both were prepared by mixing 10 µl of standard solution and 190 µl of buffer. miRNA sample measurement was realized by a combination of 198 µl of working solution and 2µl from each sample (141).

3.3.7. Detection of microRNA Expression Levels by Real-Time Polymerase Chain Reaction (RT-PCR)

The miRNAs selected for this study were determined by using the “mirbase” and “targetscan” (http://www.mirbase.org/, http://www.targetscan.org/) databases. miRNAs and their targets were analyzed using these data bases regarding molecular mechanisms in GBM.

miRNA-582-5p and miRNA-363 were chosen as the target miRNAs for this project. Primer sequences of the chosen miRNAs can be seen in Table 3.3.7-1. Comprehension of miRNA expression levels has been realized by delta C_T (ΔC_T), and internal control (housekeeping assay, RNU6) has been used for calculation of fold change. miRNA expression level determinations were accomplished using the delta delta C_T ($\Delta\Delta C_T$) and fold change equations (142).

A real-time PCR machine (Rotor Gene-Q, Qiagen) was used for miRNA expression analyses (Table 3.3.7-3).

The amount of fluorescent sparkle through the device was determined by the binding of miRNA primer sequences (miRNA-582-5p and miRNA-363, Qiagen), which were marked with Syber Green dye (miScript SYBR Green PCR, Qiagen), cDNA sequences and a housekeeping primer (mirRU6, Qiagen) (143).

Real time PCR reagents and reaction mixtures are shown in Table 3.3.7-2.

Table 3.3.7-1. miRNA Primer Sequences

microRNA	**Primer Sequence**
hsa-miR-582-5p	- UUACAGUUGUUCAACCAGUUACU-
hsa-miR-363	-UGUUGUCGGGUGGAUCACGAUGC-

Table 3.3.7-2. Ingredients of the Mixture for miRNA Expression Analysis

SYBR Green PCR Mix	12,5 µl
miScript Universal Primer	2,5 µl
miScript Primer Assay	2,5 µl
RNase free water	2,5 µl
cDNA	6 µl
Total volume:	**20 µl**

Table 3.3.7-3. miRNA Expression Analysis Cycle Conditions

Denaturation State	95°C	20 min.	
Cycling State	94°C	15 sec.	10 Cycle
	55°C	30 sec.	
	70°C	30 sec.	
Cycling State	94°C	15 sec.	45 Cycle
	55°C	30 sec.	
	70°C	30 sec.	
Melting Curve	95°C	30 sec.	

3.3.8. Detection of Serum Caspase-9 Levels by ELISA

Serum Caspase-9 level was measured using human Caspase-9 ELISA Kit of eBioscience. For this experiment, serum samples that were used were stored at -20°C. Seven standard solutions were prepared as 100 ng/ml, 50.0 ng/ml, 25.0 ng/ml, 12.5 ng/ml, 6.3 ng/ml, 3.1 ng/ml and 1.6 ng/ml. All standard solutions were distributed to the blank wells. Sample wells were host of the sample diluents which were added 50µl and also were added 50µl of each serum samples. After this process 50µl of detection antibodies were added to all of the wells. Then, the ELISA plate was covered with adhesive film and immediately placed inside of a shaker at room temperature for 2 hours. During this time, anti-rabbit-IgG-HRP was prepared according to the rules of the instructor. At the end of the 2 hours, adhesive film was removed and the microwells were washed using ELISA plate washer 3 times. After the washing steps, 100µl of anti-rabbit-IgG-HRP were added to each of the wells.

Adhesive film was again used to cover of the microplate, and it was placed into the shaker for 1 hour at room temperature. After the incubation step the liquid in the microplate was aspirated and washed 3 times using the ELISA washing apparatus. Immediately after the plate was taken from the device, 100µl of TMB substrate solution was added to each well. The microplate was covered during aluminum foil and then incubated for 10 minutes. Color change was observed with this stage, and stop solution was scattered as quickly as possible uniformly into each solution. By this way enzyme activation was inactivated. Results were read spectrophotometer set at 450 nm wavelength. Caspase-9 level was calculated in µg / ml.

3.3.9. Detection of Caspase-9 Polymorphism by Real-Time Polymerase Chain Reaction (RT-PCR)

Genotyping was performed through Polymerase Chain Reactions (RT-PCR) using the 7500 Fast-Real Time PCR (Applied Biosystems) instrument. The caspase-9 gene Ex5+32 G>A (rs1052576) polymorphism was the gene region used in the genotyping assay. "TaqMan single nucleotide polymorphism (SNP) Genotyping Assays" were used as region specific primers and probes. Caspase-9 genotyping mixture is shown in Table 3.3.9-1, and Table 3.3.9-2 shows the PCR conditions for this experiment.

RT- PCR is a unique system that holds baselines with amplification PCR. In addition to usual PCR, this system includes fluorescent dye-bound DNA probes that attach to the target region and allow for genotyping assay by reading the fluorescence sparkles, which can be seen as a result of hydrolysis of the probes by the Taq polymerase enzyme. For wildtype (wt) allele and mutant (m) allele determination, two different dyes with different wavelengths are used.

Table 3.3.9-1. Caspase-9 Genotyping PCR Mixture

Master Mix	10 µl
TaqMan Assay	0.5 µl
DNase-RNase Free Water	8.5 µl
Template DNA	1 µl
Total Volume	**20 µl**

Table 3.3.9-2. Caspase-9 Genotyping PCR Conditions

Holding	95^{o}C	10 min.
Denaturation	92^{o}C	15 sec.
Binding/Elongation	60^{o}C	1 min.

3.3.10. Statistical Analysis

Two different groups are present in this thesis study. One group includes patients diagnosed with GBM brain tumor, and other is made up of patients who are not diagnosed with this disease. These groups together are used for understanding the effect of expression levels of miRNA-582-5p and miRNA-363 on human serum caspase-9 level. Also, caspase-9 gene Ex5+32 G>A (rs1052576) polymorphism was investigated in these working groups.

Values were expressed as the mean ± standard deviation (X±SD). Chi-square and Fisher's exact tests were used to compare demographic informations.

Student's t-test was used to examine the significance of differences between the GBM patient and control groups. The miRNA expression levels were calculated with C_T, ΔC_T, $\Delta\Delta C_T$ and fold change. Altered miRNA expression levels were analyzed by student's t-test. Correlations were determined by using Pearson Correlation test.

Statistical analysis were performed with the SPSS 22.0 program (SPSS, Inc, Chicago, IL, USA). The diagnostic value of circulating miRNAs were determined by using Receiver Operator Curve (ROC) analysis. MedCalc software was used for ROC analysis with 95% confidence interval (CI). Reported *p* values significance level of $p<0.05$ was considered to indicate statistical significance.

4. RESULTS

4.1. Demographic Results of Working Groups

Comprehensive demographic results of 35 GBM patients and 36 healthy controls can be seen in Table 4.1-1.

The control group consisted of 23 male and 13 female, participants while the patient sample group consisted of 26 male and 9 female participants (Table 4.1-1).

Table 4.1-1. Demographic results of GBM patients and healthy controls

	Control (n=36)	**GBM Patient (n=35)**	***p* value**
Gender	Male / Female 63.9% / 36.1% (n=23) / (n=13)	Male / Female 74.3% / 35.7% (n=26) / (n=9)	0.344
Age (Year)	42.75±11.70	48.69±18.34	0.108

n=number of sample, X± SD (Mean ± Standard Deviation)

*The difference between the groups was analyzed by the advanced chi-square test (X^2) and the double independent sample student t-test.

Upon examination of the table above, it can be concluded that no statistical significance between the two groups was observed.

When the age (p=0.108) and gender (p=0.344) data of both groups were examined no statistical significance was found (Table 4.1-1).

When tumor locations of GBM cases are examined, it is seen that the tumors are mostly present in the temporal area (37.0%) (Table 4.1-2).

Table 4.1-2. Clinical data of GBM patients

Tumor Location	
Temporal n=13 (37.0%)	Thalamus n=4 (11.4%)
Frontal n=4 (11.4%)	Singulat n=3 (8.6%)
Parietal n=5 (14.3%)	Corpus callosum n=1 (2.9%)
Occipital n=2 (5.7%)	Pons n=1 (2.9%)
Cerebellum n=1 (2.9%)	Brainstem n=1 (2.9%)
Total ***n=35*** ***(100%)***	

n=patient number, %= percentage value based on sample group total

4.2. Caspase-9 Levels of the Study Groups

The mean serum caspase-9 level of the patient group was 10.56±5.59 mg/dl and the mean value of the control group was 10.27±1.93 mg/dl. However, no statistically significant difference was found between the groups (*p*=0.768) (Table 4.2-1).

Table 4.2-1. Serum caspase-9 levels of the patient and control group

	Control (n=36)	**GBM Patient (n=35)**	***p* value**
Caspase-9 (mg/dl) X± SD	10.27±1.93	10.56±5.59	0.768

n=number of sample, X± SD (Mean ± Standard Deviation)

*The difference between the groups was analyzed by the double independent sample student t-test.

The mean serum caspase-9 level was determined as 10.59±6.00 mg/dl for the IDH-1 mutant group, and 10.37±2.14 mg/dl for the IDH-1 wild type group (Table 4.2-2).

The significance of serum caspase-9 levels between IDH-1 mutant and IDH-1 wild type groups was investigated. However, no statistically significant results were found between these two molecular features (p=0.619) (Table 4.2-2).

Table 4.2-2. Comparison of serum caspase-9 levels for IDH-1 mutant and wild type

	Caspase-9 (mg/dl) X± SD	***p* value**
IDH-1 Mutant	10.59±6.00	0.619
IDH-1 Wild Type	10.37±2.14	

X± SD (Mean ± Standard Deviation)
*The difference between the groups was analyzed by the double independent sample student t-test.

4.3. Statistical Analysis of Caspase-9 Genotype and Allele Frequencies

Allele types of each individual within the patient and control groups were determined by the 7500 Fast real-time device. The obtained allelic discrimination plot from this tool is shown below.

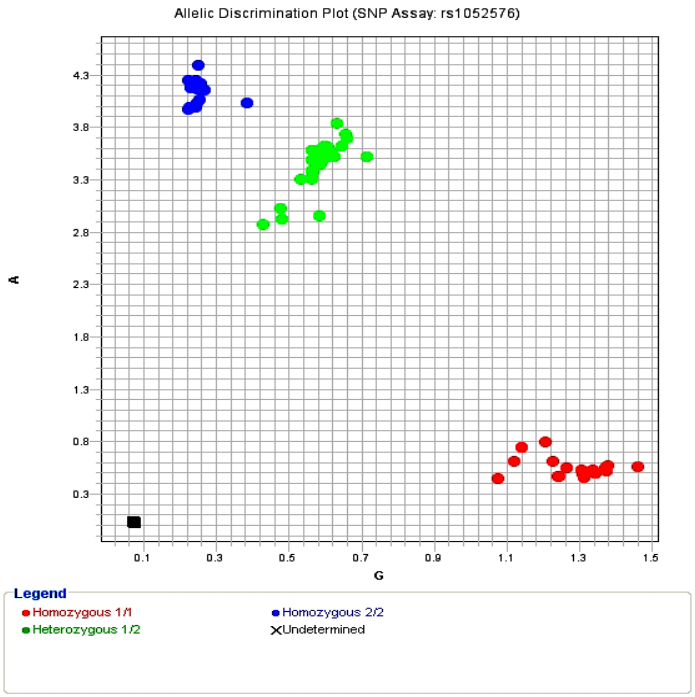


Figure 4.3-1. Allele Discrimination Display

When the characteristics of the two groups were evaluated, the frequency of the homozygous wild genotype (GG) was 33.3% in the patient group and 45.7% in the control group. Heterozygous genotype (GA) was 54.5% in the patient group and 45.7% in the control group. Homozygous mutant genotype (AA) was 12.1% in the patient group and 14.3% in the control group. There was no significant difference between the patient and control group for each homozygous GG wild type (p=0.419), heterozygous GA type (p=0.230), or homozygous AA mutant type (p=0.792) (Table 4.3-1).

40 alleles were “G” allele and 26 were “A” allele in the patient group (p=0.792). 46 alleles were “G” allele, and 24 were “A” allele in the control group (p=0.419) (Table 4.3-1).

Table 4.3-1. Caspase-9 genotype and allele distributions of patient and control groups

Genotype	**GBM Patient Group (n=33)**	**Control Group (n=35)**	***p* value**
GG	33.3% (n=11)	45.7% (n=16)	0.419
GA	54.5% (n=18)	40.0% (n=14)	0.230
AA	12.1% (n=4)	14.3% (n=5)	0.792
Allele	**Allele Count (%)**		
G	40	46	0.792
A	26	24	0.419

n= (number of sample)
*The difference between the groups was analyzed by the advanced chi-square test (X^2) and the double independent sample student t-test.

18.2% (n=6) of the patient group and 45.7% (n=16) of the control group had "GG" homozygote wild type genotype. 24.2% (n=8) of the patient group and 14.3% (n=5) of the control group had "AA" homozygote mutant genotype. 57.6% (n=19) of the patient group and 42.4% (n=14) of the control group had "GA" heterozygote genotype (Table 4.3-2).

Having a GG genotype was considered to have a protective effect 3.78 times greater than that compared to having GA or AA genotype (***p*=0.015***, 95 % CI: 1.25-11.46) for GBM patients. Also, it has been understood by the interpretation of statistical data that homozygous mutant genotype (AA) (O.R: 0.521; *p*: 0.297; 95% CI: 0.151-1.79) and heterozygous genotype (GA) (O.R: 0.491; *p*: 0.147; 95% CI: 0.187-1.291) have no risk for disease (Table 4.3-2).

Table 4.3-2. Comparison of caspase-9 genotype between patient and control groups

Genotype	GBM Patient	Control	*p* value	O.R	95% Confidence Interval
GG	(18.2%) n=6	(45.7%) n=16	**0.015***	3.78	1.25-11.46
GA+AA	(81.8%) n=27	(54.3%) n=19			
AA	(24.2%) n=8	(14.3%) n=5	0.297	0.521	0.151-1.79
GA+GG	(75.8%) n=25	(85.7%) n=30			
GA	(57.6%) n=19	(42.4%) n=14	0.147	0.491	0.187-1.291
GG+AA	(40.0%) n=14	(60.0%) n=21			

O.R (Odds Ratio)
*The difference between the groups was analyzed by the advanced chi-square test (X^2) and the double independent sample student t-test.

The relationship between serum caspase-9 level and caspase-9 genotype properties was investigated as follows:

The mean value of caspase-9 in GG genotype carriers was 10.87±2.68 mg/dl, and 9.73±4.23 mg/dl in non-carriers. No statistical significance in this genotype could be determined (*p*=0.223).

The mean value of caspase-9 in GA genotype carriers was 9.52±4.15 mg/dl, and 10.74±3.28 mg/dl in non-carriers. No statistical significance in this genotype could be determined (*p*=0.183).

The mean value of caspase-9 in AA genotype carriers was 10.56±4.91 mg/dl, and 10.10±3.57 mg/dl in non-carriers. No statistical significance in this genotype could be determined (*p*=0.733) (Table 4.3-3).

Table 4.3-3. Comparison of serum caspase-9 level and caspase-9 genotype-allele distributions of patient and control groups

Genotype		**Caspase9 (mg/dl) X± SD**	***p* value**
GG	carrier (n=26)	10.87±2.68	0.223
	non-carrier (n=42)	9.73±4.23	
GA	carrier (n=32)	9.52±4.15	0.183
	non-carrier (n=36)	10.74±3.28	
AA	carrier (n=9)	10.56±4.91	0.733
	non-carrier (n=59)	10.10±3.57	
GG	carrier	10.97±2.87	0.219
GA+AA	non-carrier	9.78±4.06	
GA	carrier	9.50±4.08	0.159
GG+AA	non-carrier	10.79±3.31	
AA	carrier	10.47±4.06	0.746
GG+AG	non-carrier	10.09±3.69	

X± SD (Mean ± Standard Deviation

*The difference between the groups was analyzed by the advanced chi-square test (X^2) and the double independent sample student t-test.

4.4. microRNA Results

C_T, ΔC_T, $\Delta\Delta C_T$ and fold change values were calculated for each of the miRNAs focused on in this thesis. These values are shown in Table 4.4.-1, Table 4.4.-2, Table 4.4.-3 and Table 4.4.-4.

The C_T values of miRNA-582-5p and miRNA-363 were examined; miRNA-582-5p means of the GBM patient and control group were 24.45±6.41 and 31.74±7.03, respectively. miRNA-363 means of the GBM patient and control group were 26.49±6.73 and 31.74±7.03, respectively (Table 4.4-1).

By comparing the C_T values, it was found that both miRNA-582-5p and miRNA-363 were downregulated in the GBM patient group. Statistically significant difference was found between GBM patient and control group (miRNA-582-5p ***p*=0.013***, 95%CI=-7.62-0.95; miRNA-363 ***p*=0.004***, 95%CI=-8.72-1.75) (Table 4.4-1).

The mean miRNA values based on ΔC_T comparison are given in Table 4.4-2. The levels of miRNA expression were determined by comparing the ΔC_T values of miRNAs and the internal control (RNU6). When the expression levels of miRNA-582-5p and miRNA-363 were compared according to the ΔC_T parameter, it was determined that the mean expression value of the patient group (miRNA-582-5p 8.50±5.85; miRNA-363 7.84±6.40) was less that of than the control group (miRNA-582-5p 12.47±6.72; miRNA-363 12.44±7.13) (Figure 4.4-1).

The expression levels of both miRNA-582-5p and miRNA-363 were found to be significantly decreased in the patient group (miRNA-582-5p ***p*=0.014***; miRNA-363 ***p*=0.010***) (Figure 4.4-1).

The $\Delta\Delta C_T$ comparisons are given in Table 4.4-3. When the expression levels of miRNA-582-5p and miRNA-363 were compared according to the $\Delta\Delta C_T$ factor, it was determined that the mean expression value of the patient group (miRNA-582-5p -2.93±5.85; miRNA-363 -4,63±6.40) was less than that of the control group (miRNA-582-5p 12.12±7.40; miRNA-363 12.10±7.39). The expression levels of both miRNA-582-5p and miRNA-363 were found to be significantly decreased in the patient group (miRNA-582-5p ***p*=0.001***; miRNA-363 ***p*=0.035***).

As in the other parameters, fold change in the expression levels of miRNA-582-5p and miRNA-363 determined using the patient group (miRNA-582- 5p 2.03±4.06; miRNA-363 3.1±4.4) and control group (miRNA-582-5p -8.40±5.1; miRNA-363-8.38±5.1) values; it was determined that miRNA-582-5p and miRNA-363 were significantly downregulated in the GBM patient group (miRNA-582-5p ***p*<0,0001***; miRNA-363 ***p*<0,0001***) (Table 4.4-4).

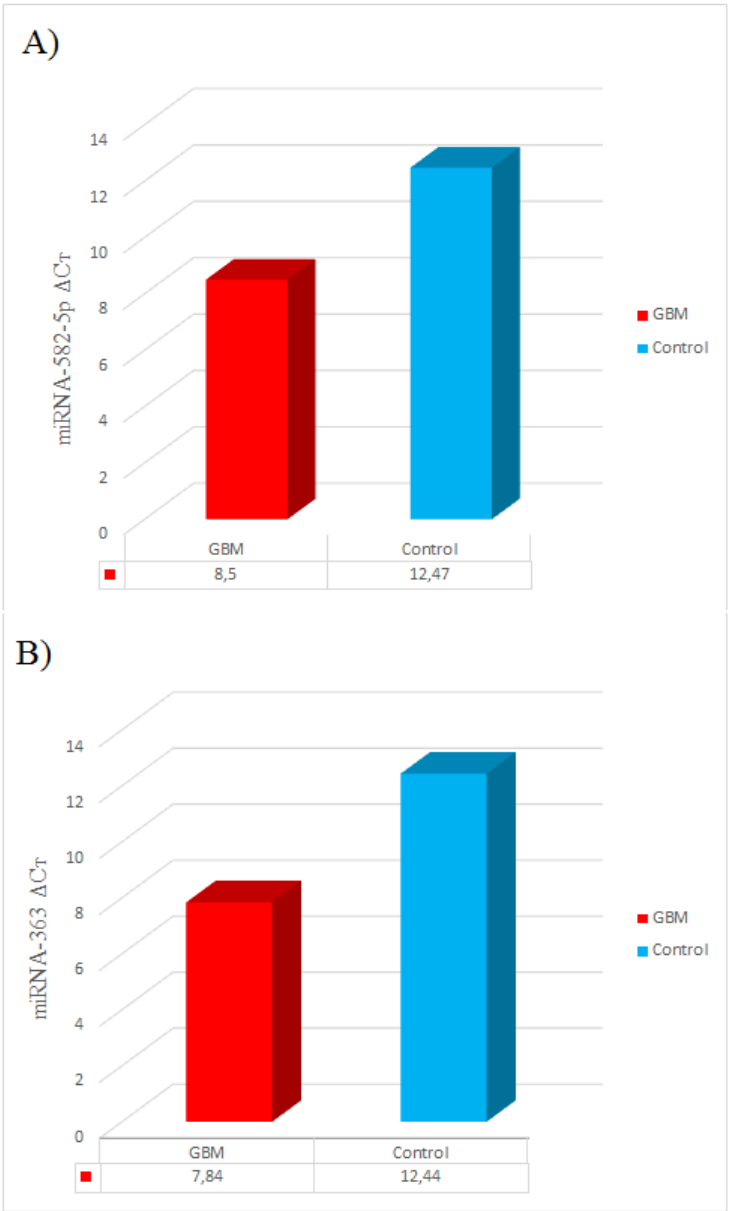


Figure 4.4-1. microRNA Analysis. **A)** Comparison of miRNA-582-5p expression levels between patient and control groups according to mean ΔCT value. **B)** Comparison of miRNA-363 expression levels between patient and control group according to mean ΔCT value.

Table 4.4-1. Comparison of C_T parameter of miRNA expression levels between patients and control groups

miRNA	Group	C_T (X± SD)	*p* value	95% Confidence Interval
miRNA-582-5p	**Patient (n=35)**	24.45±6.41	**0.013***	-7.62-0.95
	Control (n=36)	31.74±7.03		
miRNA-363	**Patient (n=35)**	26.49±6.73	**0.004***	-8.72-1.75
	Control (n=36)	31.73±7.06		

* (p<0,05). X± SD (Mean ± Standard Deviation), n (number of sample)
*The difference between the groups was analyzed by the double independent sample student t-test.

Table 4.4-2. Comparison of ΔC_T parameter of miRNA expression levels between patients and control groups

miRNA	Group	ΔC_T (X± SD)	*p* value	95% Confidence Interval
miRNA-582-5p	**Patient (n=35)**	8.50±5.85	**0.014***	-7.62-0.95
	Control (n=36)	12.47±6.72		
miRNA-363	**Patient (n=35)**	7.84±6.40	**0.010***	-8.72-1.75
	Control (n=36)	12.44±7.13		

*(p<0.05). X± SD (Mean ± Standard Deviation), n (number of sample)
*The difference between the groups was analyzed by the double independent sample student t-test.

Table 4.4-3. Comparison of $\Delta\Delta C_T$ parameter of miRNA expression levels between patients and control groups

miRNA	Group	ΔΔCT (X± SD)	*p* value	95% Confidence Interval
miRNA-582-5p	**Patient (n=35)**	-2.93±5.85	**0.001***	-18.37-11.74
	Control (n=36)	12.12±7.40		
miRNA-363	**Patient (n=35)**	-4.63±6.40	**0.035***	-20,22-13,18
	Control (n=36)	12.10±7.39		

*(p<0,05). X± SD (Mean ± Standard Deviation), n (number of sample)
*The difference between the groups was analyzed by the double independent sample student t-test.

Table 4.4-4. Comparison of fold change parameter of miRNA expression levels between patients and control groups

miRNA	Group	Fold Change (X± SD)	*p* value	95% Confidence Interval
miRNA-582-5p	**Patient (n=35)**	2.03±4.06	***p*<0.0001***	8.1-12.73
	Control (n=36)	-8.40±5.1		
miRNA-363	**Patient (n=35)**	3.1±4.4	***p*<0.0001***	9.14-14.01
	Control (n=36)	-8.38±5.1		

*(p<0,05). X± SD (Mean ± Standard Deviation), n=number of sample
*The difference between the groups was analyzed by the double independent sample student t-test.

Table 4.4-5: Comparison of serum miRNA-582-5p C_T, ΔC_T, $\Delta\Delta C_T$ and fold change levels for IDH-1 Types

Parameter	IDH-1 Type	X± SD	*p* value
miRNA-582-5p C_T	IDH-1 Mutant	27.10±6.66	0.479
	IDH-1 Wild Type	29.35±4.99	
miRNA-582-5p ΔC_T	IDH-1 Mutant	8.39±6.23	0.805
	IDH-1 Wild Type	9.11±3.58	
miRNA-582-5p $\Delta\Delta C_T$	IDH-1 Mutant	-3.04±6.23	0.805
	IDH-1 Wild Type	-2.3±3.58	
miRNA-582-5p Fold change	IDH-1 Mutant	2.10±4.32	0.805
	IDH-1 Wild Type	1.60±2.48	

X± SD (Mean ± Standard Deviation), n=number of sample
*The difference between the groups was analyzed by the double independent sample student t-test.

By comparing the C_T, ΔC_T, $\Delta\Delta C_T$ and fold change values of miRNA-582-5p, it was found that no significant relation between miRNA-582-5p expression pattern and type of IDH-1 (C_T p=0.479; ΔC_T p=0.805; $\Delta\Delta C_T$ p=0.805; fold change p=0.805) (Table 4.4-5).

Table 4.4-6. Comparison of serum miRNA-363 Cт, ΔCт, ΔΔCт and fold change levels for IDH-1 Types

Parameter	IDH-1 Type	X± SD	*p* value
miRNA-363 C_T	IDH-1 Mutant	26.72±7.00	0.619
	IDH-1 Wild Type	24.62±4.19	
miRNA-363 ΔCт	IDH-1 Mutant	8.11±6.63	0.541
	IDH-1 Wild Type	5.66±4.28	
miRNA-363 ΔΔCт	IDH-1 Mutant	-4.33±6.63	0.541
	IDH-1 Wild Type	-6.78±4.28	
miRNA-363 Fold change	IDH-1 Mutant	3.00±4.59	0.541
	IDH-1 Wild Type	4.70±2.96	

*(p<0,05). X± SD (Mean ± Standard Deviation), n=number of sample
*The difference between the groups was analyzed by the double independent sample student t-test.

The Cт, ΔCт, ΔΔCт and fold change values of miRNA-363 were calculated and compared with the IDH-1 types of the GBM patients.

No significant relation was found between miRNA-363 expression pattern and type of IDH-1 (C_T *p*=0.619; ΔCт *p*=0.541; ΔΔCт *p*=0.541; fold change *p*=0.541) (Table 4.4-6).

Table 4.4.-7. Correlation between serum caspase-9 level and miRNA C_T, ΔC_T, $\Delta\Delta C_T$ and $\ln(2^{-(\Delta\Delta CT)})$ (fold change) values of GBM patient group

Parameter	Correlation	Serum Caspase-9 Level
miRNA-582-5p C_T	Pearson Correlation *p* value	0.119
miRNA-582-5p ΔC_T	Pearson Correlation *p* value	0.144
miRNA-582-5p $\Delta\Delta C_T$	Pearson Correlation *p* value	0.885
miRNA-582-5p _ Fold Change $[\ln(2^{-(\Delta\Delta CT)})]$	Pearson Correlation *p* value	0.144
miRNA-363 C_T	Pearson Correlation *p* value	0,46
miRNA-363 ΔC_T	Pearson Correlation *p* value	0.050
miRNA-363 $\Delta\Delta C_T$	Pearson Correlation *p* value	0.050
miRNA-363_ Fold change $[\ln(2^{-(\Delta\Delta CT)})]$	Pearson Correlation *p* value	0.050

*The correlation between the groups was analyzed by the pearson correlation test.

The C_T, ΔC_T, $\Delta\Delta C_T$ and fold change values of miRNA-582-5p and miRNA-363 were calculated by correlation test and compared with the serum caspase-9 levels of the GBM patients. No significant correlation was found between miRNA-582-5p, miRNA-363 expression patterns and serum caspase-9 levels (miRNA-582-5p C_T p=0.119; ΔC_T p=0.144; $\Delta\Delta C_T$ p=0.885; fold change p=0.144 and miRNA-363 C_T p=0.46; ΔC_T p=0.050; $\Delta\Delta C_T$ p=0.050; fold change p=0.050) (Table 4.4-7).

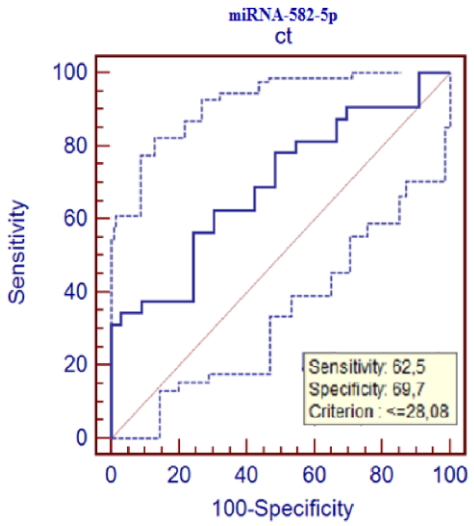
4.5. microRNA ROC Analysis

The Receiver Operating Characteristic (ROC) analysis was performed using the MedCalc Program to determine all serum miRNA levels, and the diagnostic value as well in the GBM patient and control groups.
ROC analysis which were performed with the help of MedCalc program given in Figure 4.5-1 and Figure 4.5-2.

As a result of ROC analysis, it was determined that there were significant difference between miRNA-582-5p and miRNA-363 expression levels by comparing GBM patient and control groups (miRNA582-5p fold change AUC=0.938, threshold value ≥1.66, ***p*=0.0001*** (Figure 4.5-1D); ΔΔCт, AUC= 0.939, threshold value ≤2.28, ***p*=0.0001*** (Figure 4.5-1C); ΔCт AUC=0.680, threshold value ≤11.13, ***p*=0.0066*** (Figure 4.5-1B); Cт AUC= 0.694, threshold value ≤28.08, ***p*=0.0029*** (Figure 4.5-1A) and miRNA-363 fold change AUC=0.951, threshold value >-3.78, ***p*=0.0001*** (Figure 4.5-2D); ΔΔCт AUC=0.951, threshold value ≤4.57, ***p*=0.0001*** (Figure 4.5-2C); ΔCт parameter, AUC=0.703, threshold value ≤7.67, ***p*=0.0016*** (Figure 4.5-2B); Cт, AUC= 0.723, threshold value ≤25.08, ***p*=0.0004*** (Figure 4.5-2A)).

As a result of this analysis, it was determined that miRNA-582-5p and miRNA-363 could be used as biomarkers for diagnosis of GBM in established experimental conditions.

A) miRNA 582-5p ROC Curve (Ct)

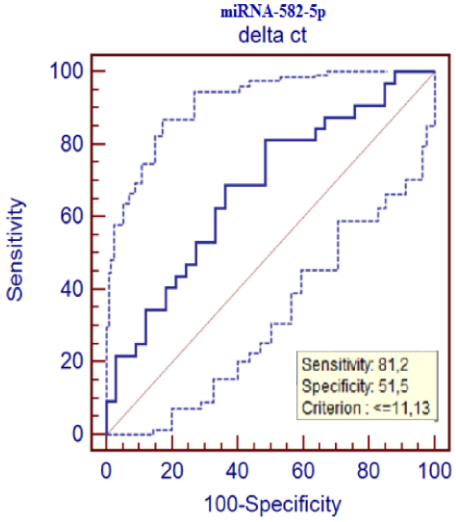


AUC=0,694

95% Cl=0,567 - 0,802

p=0,0029*

B) miRNA 582-5p ROC Curve (ΔCt)

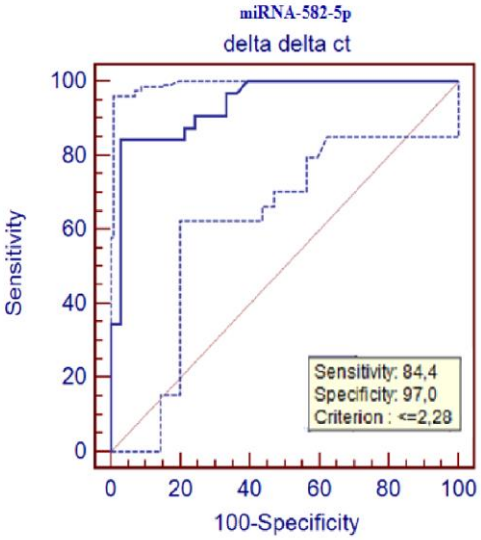


AUC=0,680

95% Cl=0,553 - 0,790

p=0,0066*

C) miRNA 582-5p ROC Curve (ΔΔCt)

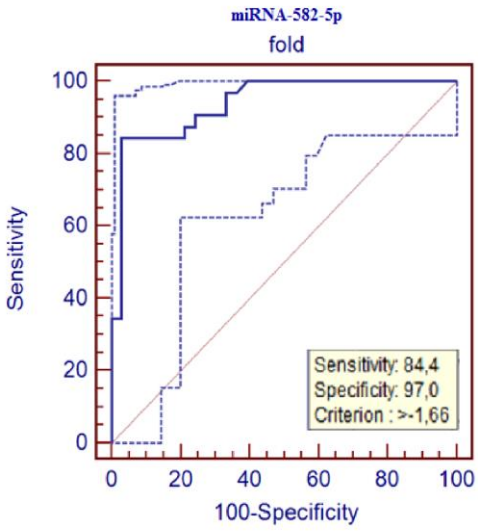


AUC=0,938

95% Cl=0,849 - 0,982

p=0,0001*

D) miRNA 582-5p ROC Curve (Fold change)



AUC=0,938

95% Cl=0,849 - 0,982

p=0,0001*

Figure 4.5-1. ROC Analysis of serum miRNA-582-5p levels of control and patient groups. A) Analysis of miRNA- 582-5p level according to C_T values. B) Analysis of miRNA-582-5p level according to ΔC_T values. C) Analysis of miRNA-582-5p level according to $\Delta\Delta C_T$ values. D) Analysis of miRNA-582-5p level according to fold change ($\ln(2^{(-\Delta\Delta C_T)})$.

* The values in the table are marked with a dark color.

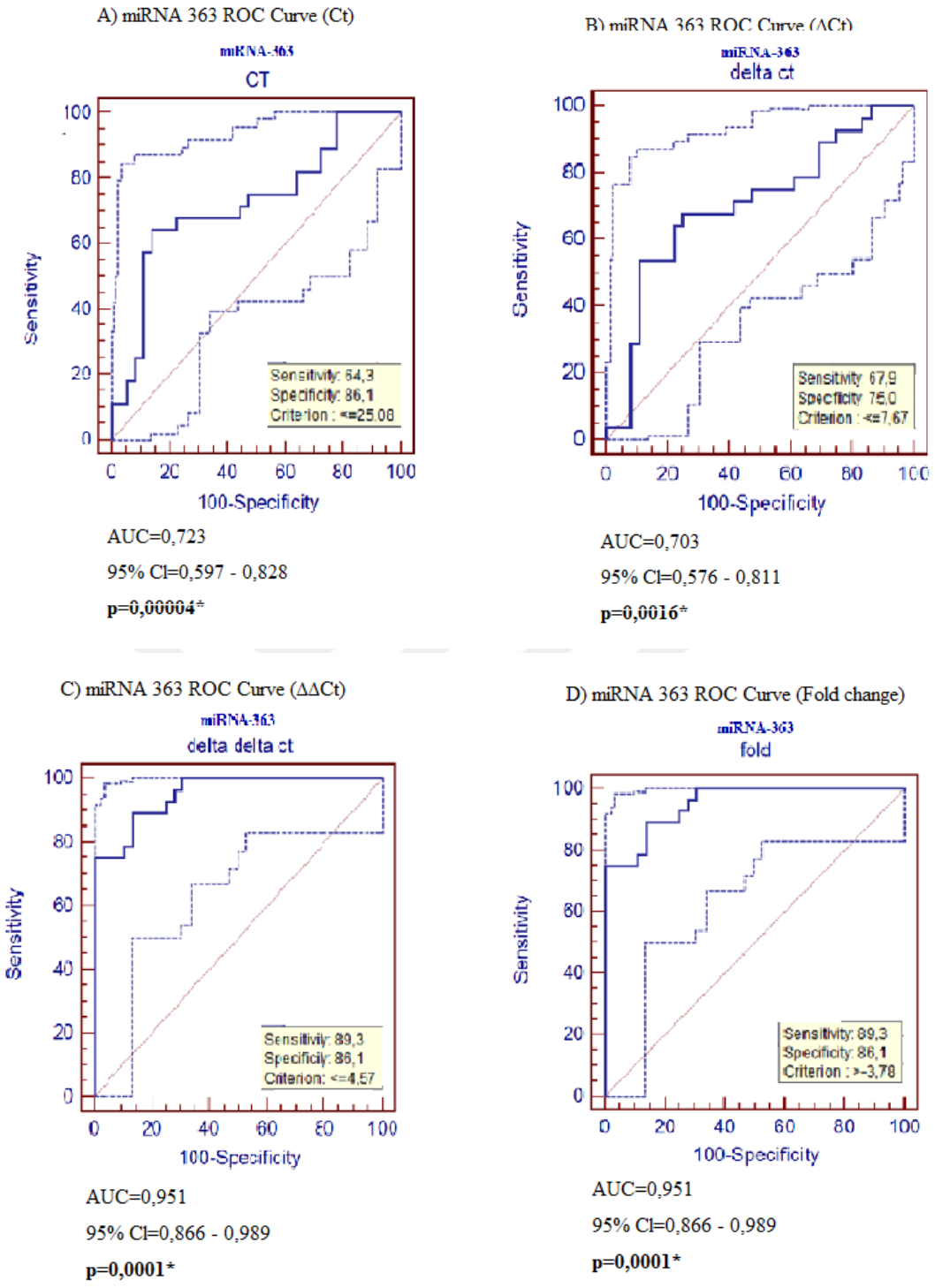


Figure 4.5-2. ROC Analysis of serum miRNA-363 levels of control and patient groups. A) Analysis of miRNA-363 level according to C_T values. B) Analysis of miRNA-363 level according to ΔC_T values. C) Analysis of miRNA-363 level according to $\Delta\Delta C_T$ values. D) Analysis of miRNA-363 level according to fold change ($\ln(2^{(-\Delta\Delta C_T)})$. * The values in the table are marked with a dark color.

5. DISCUSSION AND CONCLUSION

Glioblastoma multiforme (GBM) exhibits overwhelming tumor character with short term survival (1). According to statistical studies from the United States of America, the rate of death caused by malignant brain tumors is 22,000 cases per year, and approximately 80% of these cases are glioblastoma-induced (2). For the treatment of the disease following surgical operation, clinical applications such as radiation and chemotherapy are performed following each other (3).
In Turkish population, death caused by brain tumors take eighth and ninth places in females and males respectively (144).

MicroRNAs (miRNA) are small, non-coding, stabile RNA molecules against extreme pH and temperature conditions that are involved in many vital processes. The role of miRNAs in brain tumors has been recently discovered and is increasingly recognized (145). There is no study evaluating miRNA-582-5p and miRNA-363 effects on caspase-9 activity and caspase-9 polymorphism in Turkish patients with GBM, it was aimed to realize the present study.

In this study, all demographic data of the patient and control groups were compared in order to define the characteristics of the groups.

As summarized in Table 4.1-1, the gender (p=0.344) and age (p=0.108) factors were analyzed and no statistical significance was determined between the patient and the control group.

Glioma development can be rooted from different hemispheres depends on the glial tissue volume (146). True detection and determination of their locations are important for specific treatment strategies and survival of patients (146).

Larjavaara et al. have carried through an experiment which has showed the location of glioma and its frequency. Frontal lobe was shown as the place where a high amount of glioma was detected, while occipital lob was shown as the place where a less amount of glioma was detected (146).

Another work that supports the previous work which was achieved by Simpson et al. has shown that the gliomas have originated mostly from the frontal and temporal areas and an irregular distribution has been shown in the their study group (147).

The tumor locations of our GBM patients were evaluated and the areas where they were identified are; temporal lobe (37.0%), parietal lobe (14.3%), frontal lobe (11.4%), thalamus (11.4%), singulat (8.6%), occipital lobe(5.7%), cerebellum (2.9%), corpus callosum (2.9%), pons (2.9%) and brainstem (2.9%) (Table 4.1-2).

As one of the cysteine-aspartic protease, caspase-9 triggers the executioner caspases by cleavage and the cell death is induced (12). Various cellular stresses can trigger the intrinsic apoptosis pathway by Cyt C releasing from the mitochondria. These stages lead to the formation of apoptosome complex which includes Cyt C, caspase-9, APAF-1 and ATP. By these interactions caspase-9 can be active and apoptosis can occur (12). Kuida et al. and Hakem et al. have shown neuronal excessiveness and uncontrolled brain growth in case of deficiency of caspase-9 by animal experiment (148,149).

In this work, the mean levels of human serum caspase-9 were 10.27 mg/dl in the control group and 10.56 mg/dl in the GBM patients. Thus, serum caspase-9 levels were not statistically different among groups (p=0.768) (Table 4.2-1).

Histopathology has golden value in grade determination and diagnosis of gliomas (150). Genetical changes provide objective assessment in histopathological classification (150). Today, cytosolic $NADP^+$ related isocitrate dehydrogenase (IDH-1) and MGMT promoter side methylation are accepted as two confirmed genetic biomarkers for patients who suffer from GBM (150). In this case, determination of IDH-1 status has more importance than histologic features such as necrosis (151).

The conversion of isocitrate to alpha-ketoglutarate, which is the normal process in brain metabolism, is mediated by the IDH-1 enzyme which is the product of the IDH-1 gene (151). The use of mutation of this gene as a marker has led to prediction of life expectancy and new treatment approaches. GBM cases genetically can be classified under two groups by the assessment of IDH-1 gene. IDH-1 wild type (90%) describes primary glioblastoma cases above 55 years of age, while IDH-1 mutant (10%) gliomas have a malignant character and are usually seen in young patients (32).

In a study conducted in 2008, more than 20,000 genes were screened for IDH-1 parameter in GBMs and the mutation in this gene has been shown to be present in a small proportion of glioblastoma samples. 12% GBM cases have presented this mutation (152). A study has shown that while IDH-1 gene mutations are shown in secondary GBMs, this gene mutation has not been identified in primary GBMs (153). That is the reason why IDH-1 mutation can be accepted as a biomarker for secondary GBM (35). Based on this information, the effect of IDH-1 type difference on serum caspase-9 level was investigated in GBM patients. The mean levels of serum caspase-9 were 10.59 mg/dl in the IDH-1 mutation carriers and 10.37 mg/dl in the IDH-1 wild type group. Thus, serum caspase-9 levels were not statistically different among groups (p=0.619) (Table 4.2-1). It was detected that IDH-1 status have not effect on caspase-9 activity.

As a result of detailed screening of human miRNA genes, miRNAs have relation with some areas as cpG islands, repeat sequences, and fragile sites (154). It has been reported that cancer pathway associated miRNAs may exhibit different behaviors depending on the presence of single nucleotide polymorphism (SNP) (155). It was shown that SNPs have roles in regulation of miRNA biogenesis and functions. In this study, caspase-9 gene Ex5+32 G>A (rs1052576) polymorphism was investigated among GBM patients and undiagnosed individuals in Turkish population. In order to achieve this, 35 GBM patients were studied and compared with 36 control samples. Obtained results were evaluated statistically for each group.

All the GBM patients were examined and the most frequent genotypes the least common genotypes can be seen; GA (54.5%), GG (33.3 %), AA (12.1%) (Table 4.3-1). All the controls were examined and the most frequent genotypes the least common genotypes can be seen; GG (45.7%), GA (40.0%), AA (14.3%) (Table 4.3-1). Genotype distributions were evaluated among GBM patients and controls as wild type (p=0.419), heterozygous (p=0.230), homozygous mutant (p=0.792) and no statistical significance was determined between the patient and the control group (Table 4.3-1). GG genotype was found to have a protective effect of 3.78 times compared to the GA and AA genotypes (***p*=0.015***) (Table 4.3-2). Study that support our findings were performed by Ozdogan et al. According to the study, no statistically significant relation was found between caspase-9 gene and primary brain tumors. They have shown that GG genotype had significantly decreased in GBM (156).

Also, the mean serum caspase-9 levels and GG, GA, AA genotype carriers were determined as respectively; 10.87±68 mg/dl; 9.52±4.15 mg/dl and 10.56±4.91 mg/dl (Table 4.3-3). It was found that carrying or not carrying GG, GA and AA genotypes did not affect serum caspase-9 levels in both study groups (GG; p=0.223, GA; p=0.183, AA; p=0.733) (Table 4.3-1).

Many studies have reported that miRNAs as non invasive biomarkers can be circulated in both body fluids and solid tissues (106,115-120). Extracellular miRNAs can circulate by means of exosomes, macrovesicles, apoptotic bodies and proteins such as AGO2 (112, 113, 115).

Molecular diagnostic signs made from biologic ingredients are called biomarkers. In this context, miRNAs have become novel biomarker candidates especially in human cancer diagnosis (79, 116). The main theme of recent studies, which focus on the relationship between miRNAs and their effects on disease, are to determine whether miRNA can be a disease-specific biomarker or not.

Floyd and colleagues have completed a study on the relationship between miRNAs and their effects on caspase-9 in glioblastoma stem cells (13). miRNA-582-5p and miRNA-363 have been selected as target miRNAs in GBM cell lines. They have shown that these miRNAs show their effects by inhibiting the activities of caspase-3 and caspase-9. By this way, the apoptotic process of the cell is blocked and tumor survival is achieved (13).

Also, Conti et al., have worked with miRNA-363 in glioma cell lines. They have demonstrated that viability of glioma cells were reduced by inhibition of miRNA-363 expression. Thus, they have said that miRNA-363 can be a marker of glioma (145).

In this thesis, it is aimed to clarify the molecular mechanism of glioblastoma multiforme with a specific point of view. In this study, the biological role of miRNA-582-5p and miRNA-363 in human glioblastoma multiforme were explored by using serum samples. For this approach experiments and statistical analysis have been used.

C_T values which were obtained via RT-PCR analyses have shown that miRNA-582-5p and miRNA-363 were downregulated in GBM patient group (Table 4.4-1). Levels of miRNA-582-5p expression were 24.45 ± 6.41 in the GBM patient group, and 31.74 ± 7.03 in the control group. Levels of miRNA-363 expression were 26.49 ± 6.73 in the GBM patient group, and 31.73 ± 7.06 in the control group.

The ΔC_T, $\Delta\Delta C_T$ and fold change parameters were calculated in order to detailed evaluation of the miRNA expression levels (Table 4.4-2, 4.4-3 and Table 4.4-4).

The ΔC_T values were calculated through comparison of the obtained C_T values of the groups with the internal control (RNU6); miRNA-582-5p expression levels were 8.50±5.85 in GBM patients and 12.47±6.72 in controls. miRNA-363 expression levels were 7.84±6.40 in GBM patients and 12.44±7.13 in controls. Thus, miRNA-582-5p (***p*=0.014***) and miRNA-363 (***p*=0.010***) expression levels in GBM group were significantly downregulated (Table 4.4-2).

When $\Delta\Delta C_T$ changes are examined for each of miRNA; miRNA-582-5p expression levels were -2.93±5.85 in GBM patients and 12.12±7.40 in controls, while miRNA-363 expression levels were -4.63±6.40 in GBM patients and 12.10±7.39 in controls. Also, miRNA-582-5p (***p*=0.014***) and miRNA-363 (***p*=0.010***) expression levels in GBM group were significantly downregulated (Table 4.4-3).

Fold changes [$(\ln(2^{(-\Delta\Delta C_T)})$] are calculated through meta-controls which included in all control groups of each of miRNAs. According to all controls; miRNA-582-5p expression levels 2.03±4.06 in GBM patients and -8.40±5.1 in controls, miRNA-363 expression levels 3.1±4.4 in GBM patients and -8.38±5.1 in controls.
It has been found that miRNA-582-5p (***p*<0.0001***) and miRNA-363 (***p*<0.0001***) expression levels in GBM group were significantly downregulated (Table 4.4-4).

The effect of having an IDH-1 wild type or IDH-1 mutation on GBM pathology has been demonstrated by means of many studies (151). Using this information, it was investigated whether the presence of IDH-1 mutation or IDH-1 wild type had effects on miRNA-582-5p and miRNA-363 expression levels. The C_T, ΔC_T, $\Delta\Delta C_T$ and fold change parameters were used in order to obtain a detailed evaluation of the relation between miRNA expression levels and IDH-1 mutation/IDH-1 wild type (Table 4.4-5). The C_T, ΔC_T, $\Delta\Delta C_T$ and fold change parameters of miRNA-582-5p were evaluated and it was determined that having the IDH-1 mutation or the IDH-1 wild type had no significant affect the expression level of this miRNA (C_T p=0.479; ΔC_T p=0.805, $\Delta\Delta C_T$ p=0.805 and fold change p=0.805). The C_T, ΔC_T, $\Delta\Delta C_T$ and fold change parameters of miRNA-363 were evaluated and it was determined that having the IDH-1 mutation or the IDH-1 wild type had no significant affect the expression level of this miRNA (C_T p=0.619; ΔC_T p=0.541, $\Delta\Delta C_T$ p=0.541 and fold change p=0.541).

As a main approach, serum caspase-9 level and serum miRNA levels were examined. No correlation was found between serum miRNA-582 and miRNA-363 expression levels and serum caspase-9 level for all parameters (miRNA-582-5p C_T p=0.119; ΔC_T p=0.144, $\Delta\Delta C_T$ p=0.885, fold change p=0.144; miRNA-363 C_T p=0.46; ΔC_T p=0.050, $\Delta\Delta C_T$ p=0.050, fold change p=0.050). According to these results, it was found that there was no relationship between miRNA-582-5p and miRNA-363 expression levels and their effects on serum caspase-9 levels.

Finally, whether the target miRNAs, miRNA-582-5p and miRNA-363, were biomarkers or not for GBM, they were evaluated by the Receiver Operating Characteristic (ROC) analysis which were performed with the help of MedCalc program (Figure 4.5-1 and Figure 4.5-2).

As a result of ROC analysis, it was determined that there were significant difference between miRNA-582-5p and miRNA-363 expression levels by comparing GBM patient and control groups (miRNA582-5p fold change AUC=0.938, threshold value ≥1.66, ***p*=0.0001*** (Figure 4.5-1D); ΔΔCT, AUC= 0.939, threshold value ≤2.28, ***p*=0.0001*** (Figure 4.5-1C); ΔCT AUC=0.680, threshold value ≤11.13, ***p*=0.0066*** (Figure 4.5-1B); CT AUC= 0.694, threshold value ≤28.08, ***p*=0.0029*** (Figure 4.5-1A) and miRNA-363 fold change AUC=0.951, threshold value >-3.78, ***p*=0.0001*** (Figure 4.5-2D); ΔΔCT AUC=0.951, threshold value ≤4.57, ***p*=0.0001*** (Figure 4.5-2C); ΔCT parameter, AUC=0.703, threshold value ≤7.67, ***p*=0.0016*** (Figure 4.5-2B); CT, AUC= 0.723, threshold value ≤25.08, ***p*=0.0004*** (Figure 4.5-2A)).

As a result of this analysis, it was determined that miRNA-582-5p and miRNA-363 could be used as biomarkers for diagnosis of GBM in established experimental conditions.

As a result;

The role of miRNA-582-5p and miRNA-363 in molecular mechanisms of GBM disease has not been fully elucidated. Also, number of the studies which were performed and published by using miRNA-582-5p and miRNA-363 are very less, as well as published articles about the roles they play in the mechanism of apoptosis. Therefore, deconstructing the roles of these target miRNAs in apoptosis and determining their regulatory status may be useful in providing a noninvasive treatment strategy for individuals with GBM.

miRNAs can target multiple molecular points and can regulate their expression levels. Therefore, multifaceted researches are required to understand whether a miRNA can be disease-specific biomarker or not.

When all the obtained results are evaluated, considering that the differences in the expression of miRNAs may provide a non-invasive strategy for the diagnosis of GBM.

As a result of this thesis finding, it is believed that changes in miRNA-582-5p and miRNA-363 expression levels can be used as biomarker candidates in terms of diagnosis of GBM and development of treatment strategies. Further studies with large number of samples will clarify these results.

The main findings of our study can be summarized as follows:

(1) According to the data obtained from this thesis, miRNA-582-5p and miRNA-363 expression levels have distinctive characteristics for GBM disease according to the groups defined as patient and control. It was shown that miRNA-582-5p and miRNA-363 expression levels did not affect serum caspase-9 levels.

(2) When miRNA expression levels were examined, miRNA-582-5p and miRNA-363 levels were compared according to C_T, ΔC_T, $\Delta\Delta C_T$ and fold change values and it was found that these miRNAs were significantly less in the GBM patient group than control.

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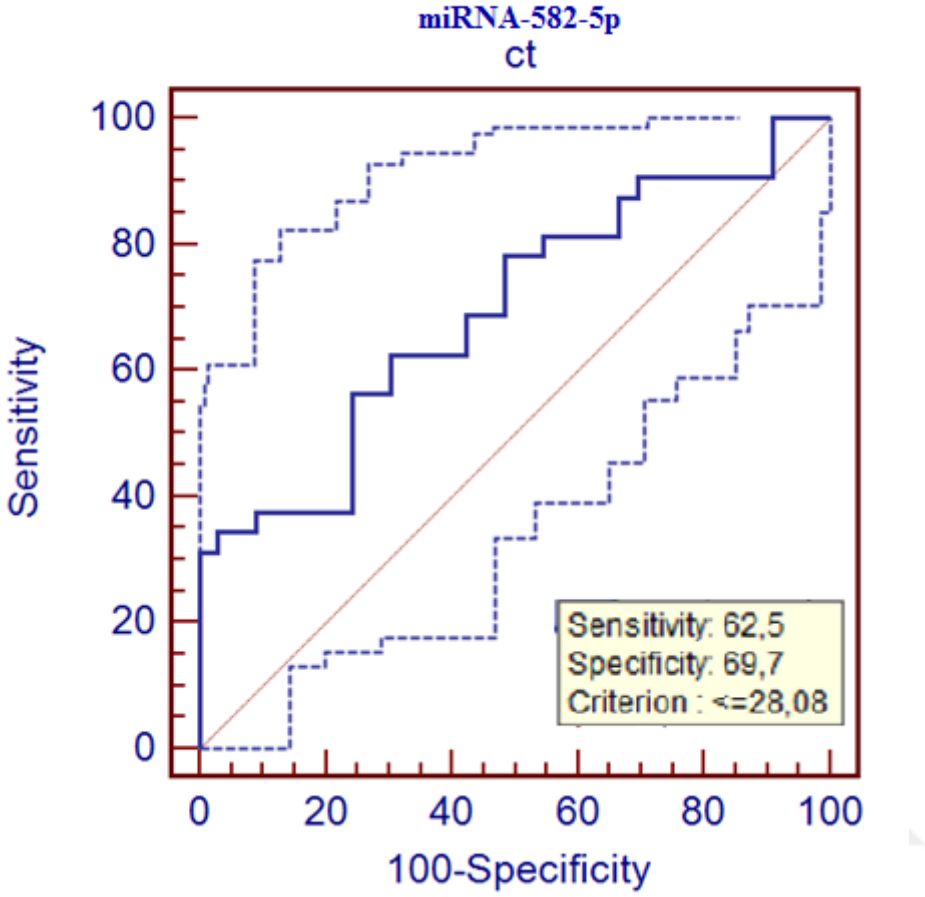
7. APPENDICES



ROC curve

Variable	miRNA-582-p_ct
Classification variable	diagnosis

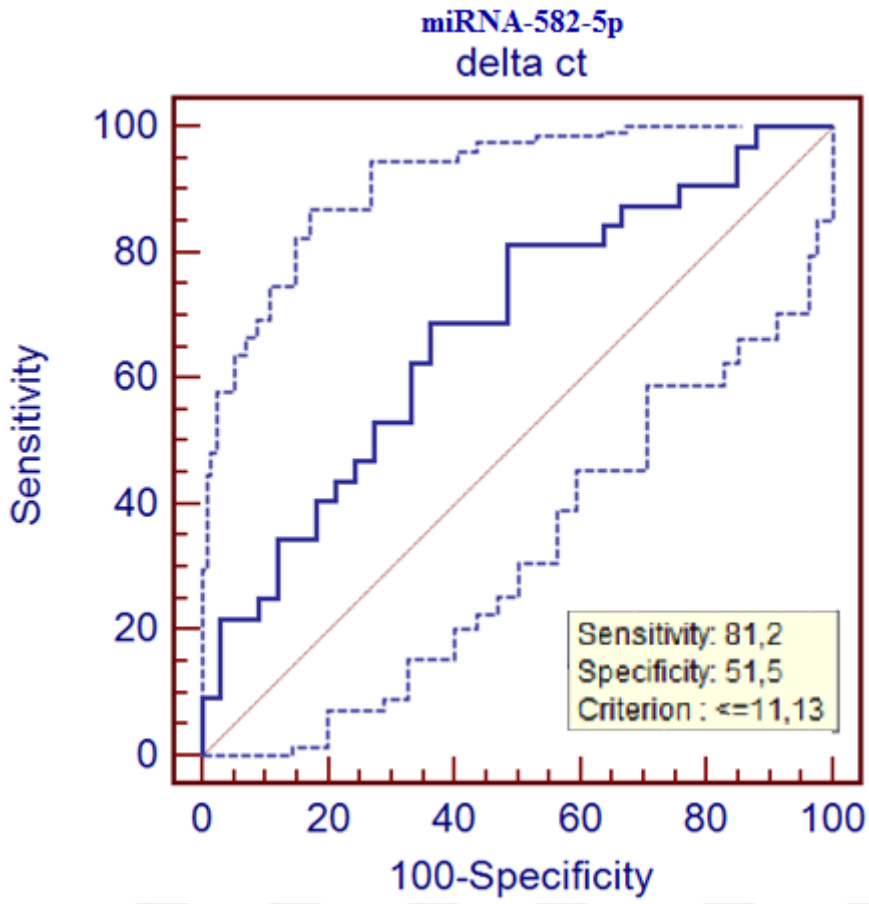
Positive group	
diagnosis	= 1
Sample size	32
Negative group	
diagnosis	= 0
Sample size	33

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,694
Standard Error	0,0653
95% Confidence Interval	0,567 to 0,802
z statistic	2,974
Significance level P (Area=0.5)	0,0029

Criterion values and coordinates of t he ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
< 20,4	0,00	0,0 - 11,0	100,00	89,3 - 100,0			1,00	
<=20,4	3,12	0,5 - 16,3	100,00	89,3 - 100,0			0,97	
<=20,46	6,25	0,9 - 20,8	100,00	89,3 - 100,0			0,94	
<=20,87	9,38	2,1 - 25,0	100,00	89,3 - 100,0			0,91	
<=21,11	12,50	3,6 - 29,0	100,00	89,3 - 100,0			0,88	
<=21,29	15,63	5,3 - 32,8	100,00	89,3 - 100,0			0,84	
<=21,6	18,75	7,3 - 36,4	100,00	89,3 - 100,0			0,81	
<=21,79	21,87	9,3 - 40,0	100,00	89,3 - 100,0			0,78	
<=21,96	25,00	11,5 - 43,4	100,00	89,3 - 100,0			0,75	
<=22,25	28,12	13,8 - 46,7	100,00	89,3 - 100,0			0,72	
<=22,39	31,25	16,1 - 50,0	100,00	89,3 - 100,0			0,69	
<=22,52	31,25	16,1 - 50,0	96,97	84,2 - 99,5	10,31	6,1 - 17,3	0,71	0,1 - 5,0
<=22,56	34,38	18,6 - 53,2	96,97	84,2 - 99,5	11,34	7,0 - 18,4	0,68	0,1 - 4,7
<=22,61	34,38	18,6 - 53,2	93,94	79,7 - 99,1	5,67	3,5 - 9,2	0,70	0,2 - 2,7
<=22,75	34,38	18,6 - 53,2	90,91	75,6 - 98,0	3,78	2,3 - 6,2	0,72	0,2 - 2,2
<=22,84	37,50	21,1 - 56,3	90,91	75,6 - 98,0	4,12	2,6 - 6,5	0,69	0,2 - 2,1
<=23,04	37,50	21,1 - 56,3	87,88	71,8 - 96,5	3,09	1,9 - 4,9	0,71	0,3 - 1,9
<=23,06	37,50	21,1 - 56,3	84,85	68,1 - 94,8	2,48	1,5 - 4,0	0,74	0,3 - 1,7
<=23,36	37,50	21,1 - 56,3	81,82	64,5 - 93,0	2,06	1,3 - 3,3	0,76	0,4 - 1,7
<=23,73	37,50	21,1 - 56,3	78,79	61,1 - 91,0	1,77	1,1 - 2,9	0,79	0,4 - 1,6
<=24,04	37,50	21,1 - 56,3	75,76	57,7 - 88,9	1,55	1,0 - 2,5	0,82	0,4 - 1,6
<=24,3	40,63	23,7 - 59,3	75,76	57,7 - 88,9	1,68	1,1 - 2,7	0,78	0,4 - 1,5
<=25,06	43,75	26,4 - 62,3	75,76	57,7 - 88,9	1,80	1,2 - 2,8	0,74	0,4 - 1,5
<=25,3	46,88	29,1 - 65,2	75,76	57,7 - 88,9	1,93	1,3 - 2,9	0,70	0,4 - 1,4
<=25,74	50,00	31,9 - 68,1	75,76	57,7 - 88,9	2,06	1,4 - 3,1	0,66	0,3 - 1,3
<=25,95	53,13	34,8 - 70,9	75,76	57,7 - 88,9	2,19	1,5 - 3,2	0,62	0,3 - 1,3
<=26,21	56,25	37,7 - 73,6	75,76	57,7 - 88,9	2,32	1,6 - 3,3	0,58	0,3 - 1,2
<=27,05	56,25	37,7 - 73,6	72,73	54,5 - 86,7	2,06	1,4 - 3,0	0,60	0,3 - 1,2
<=27,09	56,25	37,7 - 73,6	69,70	51,3 - 84,4	1,86	1,3 - 2,7	0,63	0,3 - 1,2
<=27,79	59,38	40,7 - 76,3	69,70	51,3 - 84,4	1,96	1,4 - 2,8	0,58	0,3 - 1,1
<=28,08 *	62,50	43,7 - 78,9	69,70	51,3 - 84,4	2,06	1,5 - 2,9	0,54	0,3 - 1,1
<=28,2	62,50	43,7 - 78,9	66,67	48,2 - 82,0	1,87	1,3 - 2,7	0,56	0,3 - 1,1
<=28,26	62,50	43,7 - 78,9	63,64	45,1 - 79,6	1,72	1,2 - 2,5	0,59	0,3 - 1,1
<=28,27	62,50	43,7 - 78,9	60,61	42,1 - 77,1	1,59	1,1 - 2,3	0,62	0,3 - 1,1
<=28,45	62,50	43,7 - 78,9	57,58	39,2 - 74,5	1,47	1,0 - 2,2	0,65	0,4 - 1,2
<=28,47	65,62	46,8 - 81,4	57,58	39,2 - 74,5	1,55	1,1 - 2,3	0,60	0,3 - 1,1
<=29,14	68,75	50,0 - 83,9	57,58	39,2 - 74,5	1,62	1,1 - 2,4	0,54	0,3 - 1,0
<=29,33	68,75	50,0 - 83,9	54,55	36,4 - 71,9	1,51	1,0 - 2,2	0,57	0,3 - 1,1
<=29,4	68,75	50,0 - 83,9	51,52	33,6 - 69,2	1,42	0,9 - 2,1	0,61	0,3 - 1,1
<=29,88	71,87	53,3 - 86,2	51,52	33,6 - 69,2	1,48	1,0 - 2,2	0,55	0,3 - 1,1
<=29,94	75,00	56,6 - 88,5	51,52	33,6 - 69,2	1,55	1,1 - 2,3	0,49	0,2 - 1,0
<=30,06	78,12	60,0 - 90,7	51,52	33,6 - 69,2	1,61	1,1 - 2,4	0,42	0,2 - 0,9
<=30,25	78,12	60,0 - 90,7	48,48	30,8 - 66,4	1,52	1,0 - 2,3	0,45	0,2 - 0,9
<=30,33	78,12	60,0 - 90,7	45,45	28,1 - 63,6	1,43	0,9 - 2,2	0,48	0,2 - 1,0
<=30,48	81,25	63,6 - 92,7	45,45	28,1 - 63,6	1,49	1,0 - 2,2	0,41	0,2 - 0,9
<=31,5	81,25	63,6 - 92,7	42,42	25,5 - 60,8	1,41	0,9 - 2,2	0,44	0,2 - 1,0
<=31,62	81,25	63,6 - 92,7	39,39	22,9 - 57,9	1,34	0,9 - 2,1	0,48	0,2 - 1,0
<=33,75	81,25	63,6 - 92,7	36,36	20,4 - 54,9	1,28	0,8 - 2,1	0,52	0,2 - 1,1
<=34,71	81,25	63,6 - 92,7	33,33	18,0 - 51,8	1,22	0,7 - 2,0	0,56	0,3 - 1,2
<=35,31	84,37	67,2 - 94,7	33,33	18,0 - 51,8	1,27	0,8 - 2,1	0,47	0,2 - 1,1
<=36,44	87,50	71,0 - 96,4	33,33	18,0 - 51,8	1,31	0,8 - 2,2	0,37	0,1 - 1,0
<=37,46	87,50	71,0 - 96,4	30,30	15,6 - 48,7	1,26	0,7 - 2,1	0,41	0,2 - 1,1
<=38,13	90,62	75,0 - 97,9	30,30	15,6 - 48,7	1,30	0,8 - 2,2	0,31	0,1 - 0,9
<=38,16	90,62	75,0 - 97,9	27,27	13,3 - 45,5	1,25	0,7 - 2,2	0,34	0,1 - 1,0
<=38,68	90,62	75,0 - 97,9	24,24	11,1 - 42,3	1,20	0,6 - 2,2	0,39	0,1 - 1,2
<=39,06	90,62	75,0 - 97,9	21,21	9,0 - 38,9	1,15	0,6 - 2,2	0,44	0,1 - 1,3
<=39,99	90,62	75,0 - 97,9	18,18	7,0 - 35,5	1,11	0,5 - 2,3	0,52	0,2 - 1,5
<=40,22	90,62	75,0 - 97,9	15,15	5,2 - 31,9	1,07	0,5 - 2,4	0,62	0,2 - 1,8
<=40,61	90,62	75,0 - 97,9	12,12	3,5 - 28,2	1,03	0,4 - 2,6	0,77	0,3 - 2,3
<=40,77	90,62	75,0 - 97,9	9,09	2,0 - 24,4	1,00	0,3 - 2,9	1,03	0,3 - 3,0
<=40,84	93,75	79,2 - 99,1	9,09	2,0 - 24,4	1,03	0,3 - 3,0	0,69	0,2 - 2,6
<=40,92	96,87	83,7 - 99,5	9,09	2,0 - 24,4	1,07	0,4 - 3,1	0,34	0,05 - 2,4
<=40,99	100,00	89,0 - 100,0	9,09	2,0 - 24,4	1,10	0,4 - 3,2	0,00	
<=42,1	100,00	89,0 - 100,0	6,06	0,9 - 20,3	1,06	0,3 - 4,1	0,00	
<=42,22	100,00	89,0 - 100,0	3,03	0,5 - 15,8	1,03	0,1 - 7,1	0,00	
<=44,98	100,00	89,0 - 100,0	0,00	0,0 - 10,7	1,00			



ROC curve

Variable	miRNA-582-5p_delta_ct delta ct
Classification variable	diagnosis

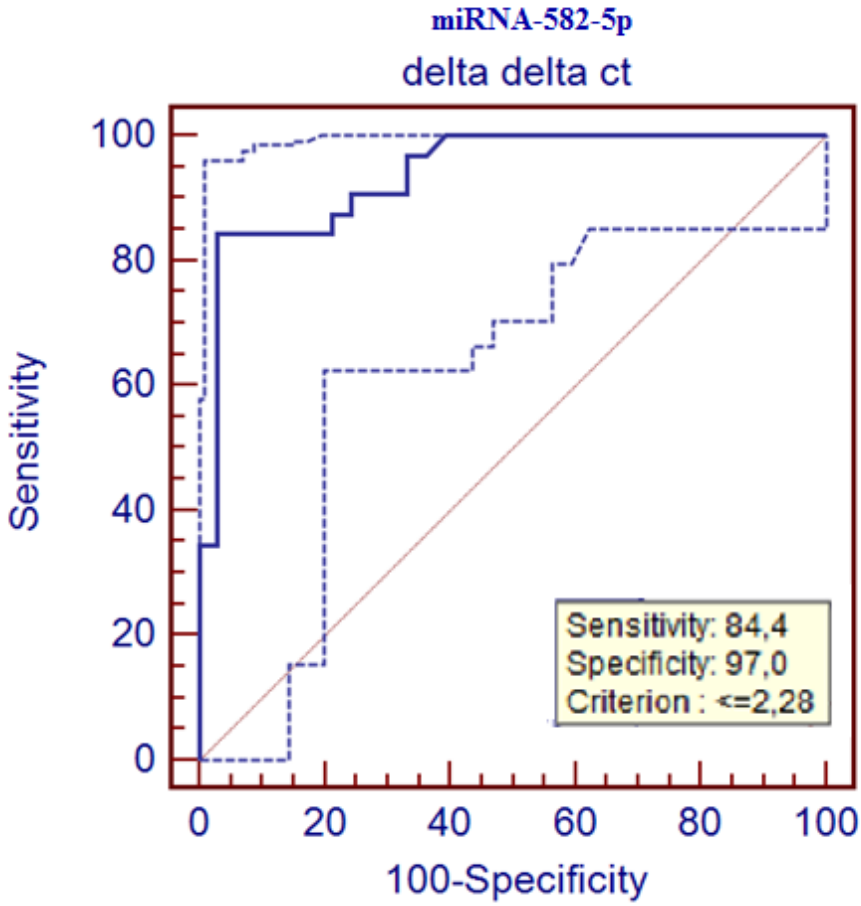
Positive group	
diagnosis	= 1
Sample size	32
Negative group	
diagnosis	= 0
Sample size	33

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,680
Standard Error	0,0663
95% Confidence Interval	0,553 to 0,790
z statistic	2,715
Significance level P (Area=0.5)	0,0066

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
< 1,52	0,00	0,0 - 11,0	100,00	89,3 - 100,0			1,00	
<=1,52	3,12	0,5 - 16,3	100,00	89,3 - 100,0			0,97	
<=2,18	6,25	0,9 - 20,8	100,00	89,3 - 100,0			0,94	
<=2,21	9,38	2,1 - 25,0	100,00	89,3 - 100,0			0,91	
<=2,39	9,38	2,1 - 25,0	96,97	84,2 - 99,5	3,09	1,1 - 9,1	0,93	0,1 - 6,5
<=2,42	12,50	3,6 - 29,0	96,97	84,2 - 99,5	4,13	1,6 - 10,3	0,90	0,1 - 6,2
<=2,73	15,63	5,3 - 32,8	96,97	84,2 - 99,5	5,16	2,3 - 11,6	0,87	0,1 - 6,0
<=2,81	18,75	7,3 - 36,4	96,97	84,2 - 99,5	6,19	3,0 - 12,8	0,84	0,1 - 5,8
<=2,85	21,87	9,3 - 40,0	96,97	84,2 - 99,5	7,22	3,7 - 13,9	0,81	0,1 - 5,6
<=3,08	21,87	9,3 - 40,0	93,94	79,7 - 99,1	3,61	1,9 - 7,0	0,83	0,2 - 3,2
<=3,21	21,87	9,3 - 40,0	90,91	75,6 - 98,0	2,41	1,2 - 4,7	0,86	0,3 - 2,6
<=3,36	25,00	11,5 - 43,4	90,91	75,6 - 98,0	2,75	1,5 - 5,1	0,83	0,3 - 2,5
<=3,52	25,00	11,5 - 43,4	87,88	71,8 - 96,5	2,06	1,1 - 3,8	0,85	0,3 - 2,2
<=3,85	28,12	13,8 - 46,7	87,88	71,8 - 96,5	2,32	1,3 - 4,1	0,82	0,3 - 2,1
<=4,17	31,25	16,1 - 50,0	87,88	71,8 - 96,5	2,58	1,5 - 4,4	0,78	0,3 - 2,0
<=4,31	34,38	18,6 - 53,2	87,88	71,8 - 96,5	2,84	1,7 - 4,7	0,75	0,3 - 1,9
<=4,4	34,38	18,6 - 53,2	84,85	68,1 - 94,8	2,27	1,4 - 3,7	0,77	0,3 - 1,8
<=4,63	34,38	18,6 - 53,2	81,82	64,5 - 93,0	1,89	1,1 - 3,1	0,80	0,4 - 1,7
<=4,66	37,50	21,1 - 56,3	81,82	64,5 - 93,0	2,06	1,3 - 3,3	0,76	0,4 - 1,7
<=4,7	40,63	23,7 - 59,3	81,82	64,5 - 93,0	2,23	1,4 - 3,5	0,73	0,3 - 1,6
<=4,89	40,63	23,7 - 59,3	78,79	61,1 - 91,0	1,92	1,2 - 3,0	0,75	0,4 - 1,5
<=6,49	43,75	26,4 - 62,3	78,79	61,1 - 91,0	2,06	1,3 - 3,2	0,71	0,3 - 1,5
<=7,26	43,75	26,4 - 62,3	75,76	57,7 - 88,9	1,80	1,2 - 2,8	0,74	0,4 - 1,5
<=7,28	46,88	29,1 - 65,2	75,76	57,7 - 88,9	1,93	1,3 - 2,9	0,70	0,4 - 1,4
<=7,78	46,88	29,1 - 65,2	72,73	54,5 - 86,7	1,72	1,1 - 2,6	0,73	0,4 - 1,4
<=7,96	50,00	31,9 - 68,1	72,73	54,5 - 86,7	1,83	1,2 - 2,7	0,69	0,4 - 1,3
<=8,02	53,13	34,8 - 70,9	72,73	54,5 - 86,7	1,95	1,3 - 2,9	0,64	0,3 - 1,3
<=8,28	53,13	34,8 - 70,9	69,70	51,3 - 84,4	1,75	1,2 - 2,6	0,67	0,4 - 1,3
<=8,45	53,13	34,8 - 70,9	66,67	48,2 - 82,0	1,59	1,1 - 2,4	0,70	0,4 - 1,3
<=8,47	56,25	37,7 - 73,6	66,67	48,2 - 82,0	1,69	1,1 - 2,5	0,66	0,4 - 1,2
<=8,64	59,38	40,7 - 76,3	66,67	48,2 - 82,0	1,78	1,2 - 2,6	0,61	0,3 - 1,2
<=8,96	62,50	43,7 - 78,9	66,67	48,2 - 82,0	1,87	1,3 - 2,7	0,56	0,3 - 1,1
<=9,27	62,50	43,7 - 78,9	63,64	45,1 - 79,6	1,72	1,2 - 2,5	0,59	0,3 - 1,1
<=9,41	65,62	46,8 - 81,4	63,64	45,1 - 79,6	1,80	1,3 - 2,6	0,54	0,3 - 1,0
<=9,71	68,75	50,0 - 83,9	63,64	45,1 - 79,6	1,89	1,3 - 2,7	0,49	0,2 - 1,0
<=9,94	68,75	50,0 - 83,9	60,61	42,1 - 77,1	1,75	1,2 - 2,5	0,52	0,3 - 1,0
<=10,16	68,75	50,0 - 83,9	57,58	39,2 - 74,5	1,62	1,1 - 2,4	0,54	0,3 - 1,0
<=10,27	68,75	50,0 - 83,9	54,55	36,4 - 71,9	1,51	1,0 - 2,2	0,57	0,3 - 1,1
<=10,46	68,75	50,0 - 83,9	51,52	33,6 - 69,2	1,42	0,9 - 2,1	0,61	0,3 - 1,1
<=10,58	71,87	53,3 - 86,2	51,52	33,6 - 69,2	1,48	1,0 - 2,2	0,55	0,3 - 1,1
<=10,78	75,00	56,6 - 88,5	51,52	33,6 - 69,2	1,55	1,1 - 2,3	0,49	0,2 - 1,0
<=11,13 *	81,25	63,6 - 92,7	51,52	33,6 - 69,2	1,68	1,2 - 2,4	0,36	0,2 - 0,8
<=11,52	81,25	63,6 - 92,7	48,48	30,8 - 66,4	1,58	1,1 - 2,3	0,39	0,2 - 0,9
<=11,86	81,25	63,6 - 92,7	45,45	28,1 - 63,6	1,49	1,0 - 2,2	0,41	0,2 - 0,9
<=12,05	81,25	63,6 - 92,7	42,42	25,5 - 60,8	1,41	0,9 - 2,2	0,44	0,2 - 1,0
<=12,84	81,25	63,6 - 92,7	39,39	22,9 - 57,9	1,34	0,9 - 2,1	0,48	0,2 - 1,0
<=13,02	81,25	63,6 - 92,7	36,36	20,4 - 54,9	1,28	0,8 - 2,1	0,52	0,2 - 1,1
<=13,71	84,37	67,2 - 94,7	36,36	20,4 - 54,9	1,33	0,8 - 2,1	0,43	0,2 - 1,0
<=15,44	84,37	67,2 - 94,7	33,33	18,0 - 51,8	1,27	0,8 - 2,1	0,47	0,2 - 1,1
<=17,13	87,50	71,0 - 96,4	33,33	18,0 - 51,8	1,31	0,8 - 2,2	0,37	0,1 - 1,0
<=17,25	87,50	71,0 - 96,4	30,30	15,6 - 48,7	1,26	0,7 - 2,1	0,41	0,2 - 1,1
<=18,82	87,50	71,0 - 96,4	24,24	11,1 - 42,3	1,15	0,6 - 2,1	0,52	0,2 - 1,3
<=18,99	90,62	75,0 - 97,9	24,24	11,1 - 42,3	1,20	0,6 - 2,2	0,39	0,1 - 1,2
<=19,09	90,62	75,0 - 97,9	21,21	9,0 - 38,9	1,15	0,6 - 2,2	0,44	0,1 - 1,3
<=19,63	90,62	75,0 - 97,9	18,18	7,0 - 35,5	1,11	0,5 - 2,3	0,52	0,2 - 1,5
<=20,09	90,62	75,0 - 97,9	15,15	5,2 - 31,9	1,07	0,5 - 2,4	0,62	0,2 - 1,8
<=20,21	93,75	79,2 - 99,1	15,15	5,2 - 31,9	1,10	0,5 - 2,5	0,41	0,1 - 1,6
<=20,38	96,87	83,7 - 99,5	15,15	5,2 - 31,9	1,14	0,5 - 2,6	0,21	0,03 - 1,4
<=21,34	96,87	83,7 - 99,5	12,12	3,5 - 28,2	1,10	0,4 - 2,8	0,26	0,04 - 1,8
<=21,37	100,00	89,0 - 100,0	12,12	3,5 - 28,2	1,14	0,5 - 2,9	0,00	
<=21,62	100,00	89,0 - 100,0	9,09	2,0 - 24,4	1,10	0,4 - 3,2	0,00	
<=21,73	100,00	89,0 - 100,0	6,06	0,9 - 20,3	1,06	0,3 - 4,1	0,00	
<=23,34	100,00	89,0 - 100,0	3,03	0,5 - 15,8	1,03	0,1 - 7,1	0,00	
<=25,2	100,00	89,0 - 100,0	0,00	0,0 - 10,7	1,00			



ROC curve

Variable	miRNA-582-5p_delta_delta_ct delta delta ct
Classification variable	diagnosis

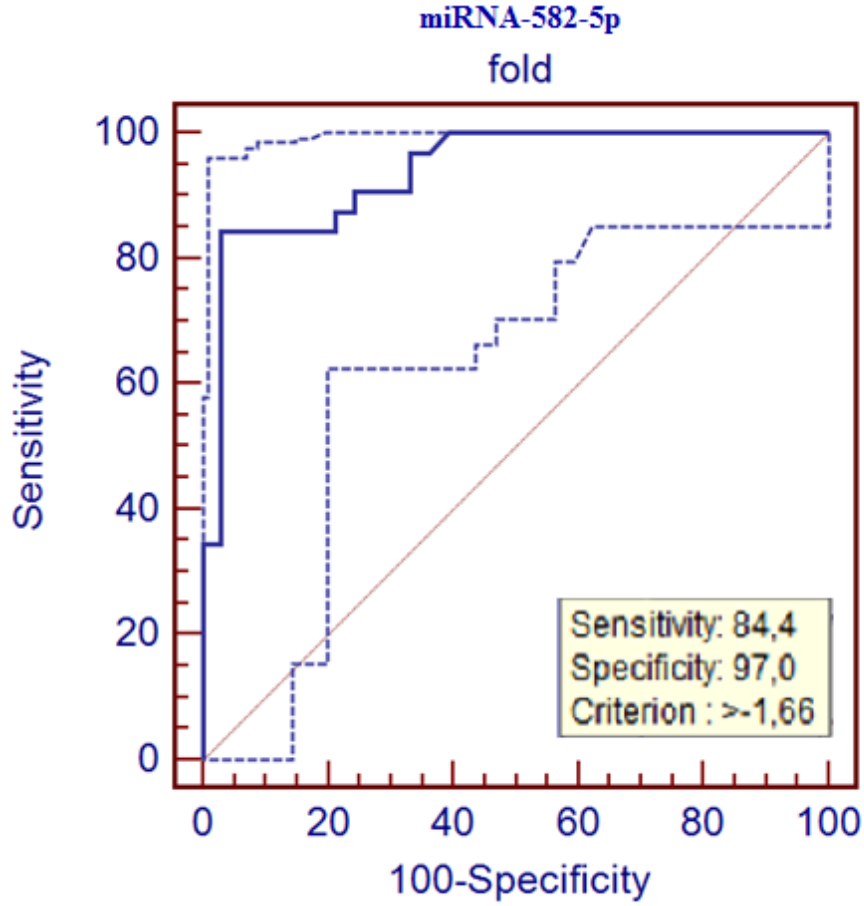
Positive group	
diagnosis	= 1
Sample size	32
Negative group	
diagnosis	= 0
Sample size	33

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,938
Standard Error	0,0314
95% Confidence Interval	0,849 to 0,982
z statistic	13,957
Significance level P (Area=0.5)	0,0001

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
< -9,91	0,00	0,0 - 11,0	100,00	89,3 - 100,0			1,00	
<=-9,91	3,12	0,5 - 16,3	100,00	89,3 - 100,0			0,97	
<=-9,25	6,25	0,9 - 20,8	100,00	89,3 - 100,0			0,94	
<=-9,22	9,38	2,1 - 25,0	100,00	89,3 - 100,0			0,91	
<=-9,01	12,50	3,6 - 29,0	100,00	89,3 - 100,0			0,88	
<=-8,7	15,63	5,3 - 32,8	100,00	89,3 - 100,0			0,84	
<=-8,62	18,75	7,3 - 36,4	100,00	89,3 - 100,0			0,81	
<=-8,58	21,87	9,3 - 40,0	100,00	89,3 - 100,0			0,78	
<=-8,07	25,00	11,5 - 43,4	100,00	89,3 - 100,0			0,75	
<=-7,58	28,12	13,8 - 46,7	100,00	89,3 - 100,0			0,72	
<=-7,26	31,25	16,1 - 50,0	100,00	89,3 - 100,0			0,69	
<=-7,12	34,38	18,6 - 53,2	100,00	89,3 - 100,0			0,66	
<=-6,8	34,38	18,6 - 53,2	96,97	84,2 - 99,5	11,34	7,0 - 18,4	0,68	0,1 - 4,7
<=-6,77	37,50	21,1 - 56,3	96,97	84,2 - 99,5	12,38	7,9 - 19,4	0,64	0,09 - 4,5
<=-6,73	40,63	23,7 - 59,3	96,97	84,2 - 99,5	13,41	8,8 - 20,5	0,61	0,09 - 4,3
<=-4,94	43,75	26,4 - 62,3	96,97	84,2 - 99,5	14,44	9,7 - 21,5	0,58	0,08 - 4,1
<=-4,15	46,88	29,1 - 65,2	96,97	84,2 - 99,5	15,47	10,6 - 22,5	0,55	0,08 - 3,9
<=-3,47	50,00	31,9 - 68,1	96,97	84,2 - 99,5	16,50	11,6 - 23,5	0,52	0,07 - 3,7
<=-3,41	53,13	34,8 - 70,9	96,97	84,2 - 99,5	17,53	12,6 - 24,4	0,48	0,07 - 3,4
<=-2,96	56,25	37,7 - 73,6	96,97	84,2 - 99,5	18,56	13,6 - 25,3	0,45	0,06 - 3,2
<=-2,79	59,38	40,7 - 76,3	96,97	84,2 - 99,5	19,59	14,6 - 26,3	0,42	0,06 - 3,0
<=-2,47	62,50	43,7 - 78,9	96,97	84,2 - 99,5	20,63	15,7 - 27,2	0,39	0,05 - 2,8
<=-2,02	65,62	46,8 - 81,4	96,97	84,2 - 99,5	21,66	16,7 - 28,0	0,35	0,05 - 2,6
<=-1,72	68,75	50,0 - 83,9	96,97	84,2 - 99,5	22,69	17,8 - 28,9	0,32	0,04 - 2,4
<=-0,85	71,87	53,3 - 86,2	96,97	84,2 - 99,5	23,72	18,9 - 29,7	0,29	0,04 - 2,2
<=-0,65	75,00	56,6 - 88,5	96,97	84,2 - 99,5	24,75	20,1 - 30,5	0,26	0,03 - 1,9
<=-0,3	81,25	63,6 - 92,7	96,97	84,2 - 99,5	26,81	22,5 - 32,0	0,19	0,02 - 1,5
<=2,28 *	84,37	67,2 - 94,7	96,97	84,2 - 99,5	27,84	23,7 - 32,7	0,16	0,02 - 1,3
<=2,39	84,37	67,2 - 94,7	93,94	79,7 - 99,1	13,92	11,7 - 16,5	0,17	0,03 - 0,8
<=3,08	84,37	67,2 - 94,7	90,91	75,6 - 98,0	9,28	7,7 - 11,2	0,17	0,04 - 0,7
<=3,21	84,37	67,2 - 94,7	87,88	71,8 - 96,5	6,96	5,7 - 8,5	0,18	0,05 - 0,6
<=3,52	84,37	67,2 - 94,7	84,85	68,1 - 94,8	5,57	4,5 - 6,9	0,18	0,06 - 0,6
<=4,4	84,37	67,2 - 94,7	81,82	64,5 - 93,0	4,64	3,7 - 5,8	0,19	0,06 - 0,6
<=4,89	84,37	67,2 - 94,7	78,79	61,1 - 91,0	3,98	3,2 - 5,0	0,20	0,07 - 0,6
<=5,7	87,50	71,0 - 96,4	78,79	61,1 - 91,0	4,12	3,3 - 5,1	0,16	0,05 - 0,5
<=7,26	87,50	71,0 - 96,4	75,76	57,7 - 88,9	3,61	2,9 - 4,6	0,17	0,06 - 0,5
<=7,56	90,62	75,0 - 97,9	75,76	57,7 - 88,9	3,74	3,0 - 4,7	0,12	0,04 - 0,4
<=7,78	90,62	75,0 - 97,9	72,73	54,5 - 86,7	3,32	2,6 - 4,2	0,13	0,04 - 0,4
<=8,28	90,62	75,0 - 97,9	69,70	51,3 - 84,4	2,99	2,3 - 3,8	0,13	0,04 - 0,4
<=8,45	90,62	75,0 - 97,9	66,67	48,2 - 82,0	2,72	2,1 - 3,5	0,14	0,04 - 0,5
<=8,78	93,75	79,2 - 99,1	66,67	48,2 - 82,0	2,81	2,2 - 3,6	0,094	0,02 - 0,4
<=8,95	96,87	83,7 - 99,5	66,67	48,2 - 82,0	2,91	2,3 - 3,7	0,047	0,006 - 0,3
<=9,27	96,87	83,7 - 99,5	63,64	45,1 - 79,6	2,66	2,0 - 3,5	0,049	0,007 - 0,4
<=9,94	100,00	89,0 - 100,0	60,61	42,1 - 77,1	2,54	1,9 - 3,3	0,00	
<=10,16	100,00	89,0 - 100,0	57,58	39,2 - 74,5	2,36	1,8 - 3,2	0,00	
<=10,27	100,00	89,0 - 100,0	54,55	36,4 - 71,9	2,20	1,6 - 3,0	0,00	
<=10,46	100,00	89,0 - 100,0	51,52	33,6 - 69,2	2,06	1,5 - 2,9	0,00	
<=11,52	100,00	89,0 - 100,0	48,48	30,8 - 66,4	1,94	1,4 - 2,8	0,00	
<=11,86	100,00	89,0 - 100,0	45,45	28,1 - 63,6	1,83	1,3 - 2,7	0,00	
<=12,05	100,00	89,0 - 100,0	42,42	25,5 - 60,8	1,74	1,2 - 2,6	0,00	
<=12,84	100,00	89,0 - 100,0	39,39	22,9 - 57,9	1,65	1,1 - 2,5	0,00	
<=13,02	100,00	89,0 - 100,0	36,36	20,4 - 54,9	1,57	1,0 - 2,5	0,00	
<=15,44	100,00	89,0 - 100,0	33,33	18,0 - 51,8	1,50	0,9 - 2,4	0,00	
<=17,25	100,00	89,0 - 100,0	30,30	15,6 - 48,7	1,43	0,9 - 2,4	0,00	
<=18,82	100,00	89,0 - 100,0	24,24	11,1 - 42,3	1,32	0,7 - 2,4	0,00	
<=19,09	100,00	89,0 - 100,0	21,21	9,0 - 38,9	1,27	0,7 - 2,4	0,00	
<=19,63	100,00	89,0 - 100,0	18,18	7,0 - 35,5	1,22	0,6 - 2,5	0,00	
<=20,09	100,00	89,0 - 100,0	15,15	5,2 - 31,9	1,18	0,5 - 2,6	0,00	
<=21,34	100,00	89,0 - 100,0	12,12	3,5 - 28,2	1,14	0,5 - 2,9	0,00	
<=21,62	100,00	89,0 - 100,0	9,09	2,0 - 24,4	1,10	0,4 - 3,2	0,00	
<=21,73	100,00	89,0 - 100,0	6,06	0,9 - 20,3	1,06	0,3 - 4,1	0,00	
<=23,34	100,00	89,0 - 100,0	3,03	0,5 - 15,8	1,03	0,1 - 7,1	0,00	
<=25,2	100,00	89,0 - 100,0	0,00	0,0 - 10,7	1,00			



ROC curve

Variable	miRNA-582-5p_fold change
Classification variable	diagnosis

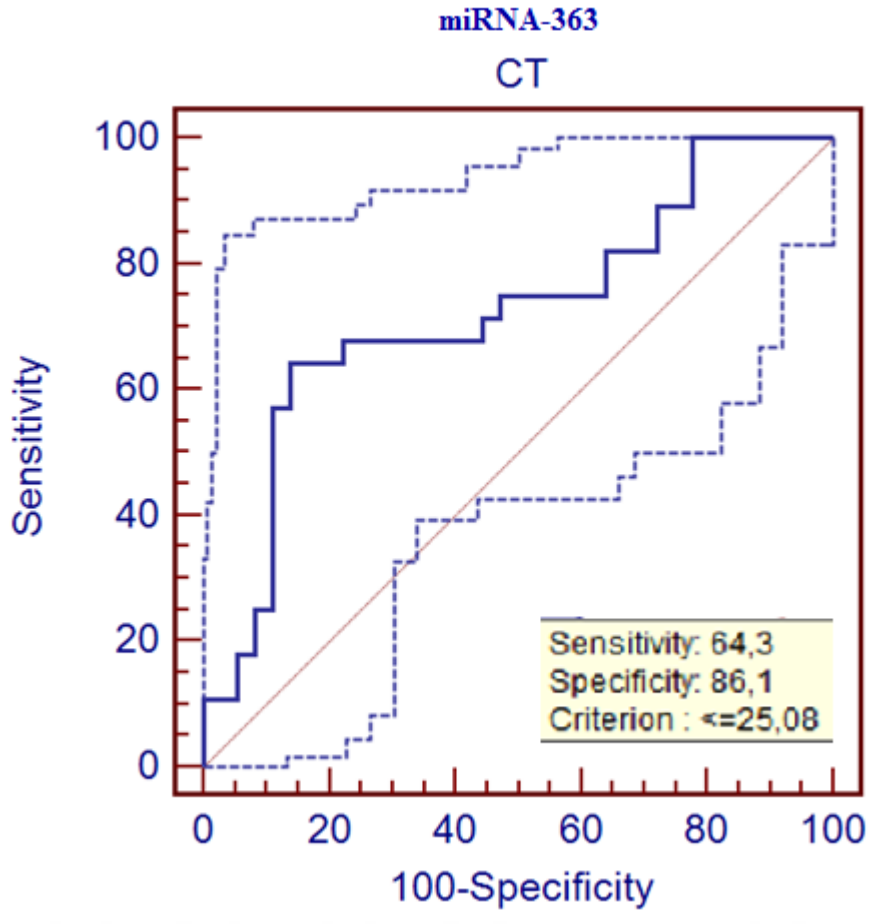
Positive group	
diagnosis	= 1
Sample size	32
Negative group	
diagnosis	= 0
Sample size	33

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,938
Standard Error	0,0318
95% Confidence Interval	0,849 to 0,982
z statistic	13,794
Significance level P (Area=0.5)	0,0001

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
>=-17,47	100,00	89,0 - 100,0	0,00	0,0 - 10,7	1,00			
>-17,47	100,00	89,0 - 100,0	3,03	0,5 - 15,8	1,03	0,1 - 7,1	0,00	
>-16,18	100,00	89,0 - 100,0	6,06	0,9 - 20,3	1,06	0,3 - 4,1	0,00	
>-15,06	100,00	89,0 - 100,0	9,09	2,0 - 24,4	1,10	0,4 - 3,2	0,00	
>-14,99	100,00	89,0 - 100,0	12,12	3,5 - 28,2	1,14	0,5 - 2,9	0,00	
>-14,79	100,00	89,0 - 100,0	15,15	5,2 - 31,9	1,18	0,5 - 2,6	0,00	
>-13,93	100,00	89,0 - 100,0	18,18	7,0 - 35,5	1,22	0,6 - 2,5	0,00	
>-13,61	100,00	89,0 - 100,0	21,21	9,0 - 38,9	1,27	0,7 - 2,4	0,00	
>-13,23	100,00	89,0 - 100,0	24,24	11,1 - 42,3	1,32	0,7 - 2,4	0,00	
>-13,05	100,00	89,0 - 100,0	30,30	15,6 - 48,7	1,43	0,9 - 2,4	0,00	
>-11,96	100,00	89,0 - 100,0	33,33	18,0 - 51,8	1,50	0,9 - 2,4	0,00	
>-10,7	100,00	89,0 - 100,0	36,36	20,4 - 54,9	1,57	1,0 - 2,5	0,00	
>-9,02	100,00	89,0 - 100,0	39,39	22,9 - 57,9	1,65	1,1 - 2,5	0,00	
>-8,9	100,00	89,0 - 100,0	42,42	25,5 - 60,8	1,74	1,2 - 2,6	0,00	
>-8,35	100,00	89,0 - 100,0	45,45	28,1 - 63,6	1,83	1,3 - 2,7	0,00	
>-8,22	100,00	89,0 - 100,0	48,48	30,8 - 66,4	1,94	1,4 - 2,8	0,00	
>-7,99	100,00	89,0 - 100,0	51,52	33,6 - 69,2	2,06	1,5 - 2,9	0,00	
>-7,25	100,00	89,0 - 100,0	54,55	36,4 - 71,9	2,20	1,6 - 3,0	0,00	
>-7,12	100,00	89,0 - 100,0	57,58	39,2 - 74,5	2,36	1,8 - 3,2	0,00	
>-7,04	100,00	89,0 - 100,0	60,61	42,1 - 77,1	2,54	1,9 - 3,3	0,00	
>-6,89	96,87	83,7 - 99,5	63,64	45,1 - 79,6	2,66	2,0 - 3,5	0,049	0,007 - 0,4
>-6,43	96,87	83,7 - 99,5	66,67	48,2 - 82,0	2,91	2,3 - 3,7	0,047	0,006 - 0,3
>-6,2	93,75	79,2 - 99,1	66,67	48,2 - 82,0	2,81	2,2 - 3,6	0,094	0,02 - 0,4
>-6,08	90,62	75,0 - 97,9	66,67	48,2 - 82,0	2,72	2,1 - 3,5	0,14	0,04 - 0,5
>-5,86	90,62	75,0 - 97,9	69,70	51,3 - 84,4	2,99	2,3 - 3,8	0,13	0,04 - 0,4
>-5,74	90,62	75,0 - 97,9	72,73	54,5 - 86,7	3,32	2,6 - 4,2	0,13	0,04 - 0,4
>-5,39	90,62	75,0 - 97,9	75,76	57,7 - 88,9	3,74	3,0 - 4,7	0,12	0,04 - 0,4
>-5,24	87,50	71,0 - 96,4	75,76	57,7 - 88,9	3,61	2,9 - 4,6	0,17	0,06 - 0,5
>-5,03	87,50	71,0 - 96,4	78,79	61,1 - 91,0	4,12	3,3 - 5,1	0,16	0,05 - 0,5
>-3,95	84,37	67,2 - 94,7	78,79	61,1 - 91,0	3,98	3,2 - 5,0	0,20	0,07 - 0,6
>-3,39	84,37	67,2 - 94,7	81,82	64,5 - 93,0	4,64	3,7 - 5,8	0,19	0,06 - 0,6
>-3,05	84,37	67,2 - 94,7	84,85	68,1 - 94,8	5,57	4,5 - 6,9	0,18	0,06 - 0,6
>-2,44	84,37	67,2 - 94,7	87,88	71,8 - 96,5	6,96	5,7 - 8,5	0,18	0,05 - 0,6
>-2,23	84,37	67,2 - 94,7	90,91	75,6 - 98,0	9,28	7,7 - 11,2	0,17	0,04 - 0,7
>-2,13	84,37	67,2 - 94,7	93,94	79,7 - 99,1	13,92	11,7 - 16,5	0,17	0,03 - 0,8
>-1,66 *	84,37	67,2 - 94,7	96,97	84,2 - 99,5	27,84	23,7 - 32,7	0,16	0,02 - 1,3
>-1,58	81,25	63,6 - 92,7	96,97	84,2 - 99,5	26,81	22,5 - 32,0	0,19	0,02 - 1,5
>0,21	75,00	56,6 - 88,5	96,97	84,2 - 99,5	24,75	20,1 - 30,5	0,26	0,03 - 1,9
>0,45	71,87	53,3 - 86,2	96,97	84,2 - 99,5	23,72	18,9 - 29,7	0,29	0,04 - 2,2
>0,59	68,75	50,0 - 83,9	96,97	84,2 - 99,5	22,69	17,8 - 28,9	0,32	0,04 - 2,4
>1,2	65,62	46,8 - 81,4	96,97	84,2 - 99,5	21,66	16,7 - 28,0	0,35	0,05 - 2,6
>1,4	62,50	43,7 - 78,9	96,97	84,2 - 99,5	20,63	15,7 - 27,2	0,39	0,05 - 2,8
>1,72	59,38	40,7 - 76,3	96,97	84,2 - 99,5	19,59	14,6 - 26,3	0,42	0,06 - 3,0
>1,94	56,25	37,7 - 73,6	96,97	84,2 - 99,5	18,56	13,6 - 25,3	0,45	0,06 - 3,2
>2,05	53,13	34,8 - 70,9	96,97	84,2 - 99,5	17,53	12,6 - 24,4	0,48	0,07 - 3,4
>2,37	50,00	31,9 - 68,1	96,97	84,2 - 99,5	16,50	11,6 - 23,5	0,52	0,07 - 3,7
>2,41	46,88	29,1 - 65,2	96,97	84,2 - 99,5	15,47	10,6 - 22,5	0,55	0,08 - 3,9
>2,88	43,75	26,4 - 62,3	96,97	84,2 - 99,5	14,44	9,7 - 21,5	0,58	0,08 - 4,1
>3,43	40,63	23,7 - 59,3	96,97	84,2 - 99,5	13,41	8,8 - 20,5	0,61	0,09 - 4,3
>4,67	37,50	21,1 - 56,3	96,97	84,2 - 99,5	12,38	7,9 - 19,4	0,64	0,09 - 4,5
>4,7	34,38	18,6 - 53,2	96,97	84,2 - 99,5	11,34	7,0 - 18,4	0,68	0,1 - 4,7
>4,72	34,38	18,6 - 53,2	100,00	89,3 - 100,0			0,66	
>4,94	31,25	16,1 - 50,0	100,00	89,3 - 100,0			0,69	
>5,04	28,12	13,8 - 46,7	100,00	89,3 - 100,0			0,72	
>5,26	25,00	11,5 - 43,4	100,00	89,3 - 100,0			0,75	
>5,6	21,87	9,3 - 40,0	100,00	89,3 - 100,0			0,78	
>5,95	18,75	7,3 - 36,4	100,00	89,3 - 100,0			0,81	
>5,98	15,63	5,3 - 32,8	100,00	89,3 - 100,0			0,84	
>6,03	12,50	3,6 - 29,0	100,00	89,3 - 100,0			0,88	
>6,25	9,38	2,1 - 25,0	100,00	89,3 - 100,0			0,91	
>6,39	6,25	0,9 - 20,8	100,00	89,3 - 100,0			0,94	
>6,41	3,12	0,5 - 16,3	100,00	89,3 - 100,0			0,97	
>6,87	0,00	0,0 - 11,0	100,00	89,3 - 100,0			1,00	



curve

Variable	miRNA-363_CT
Classification variable	Diagnosisi

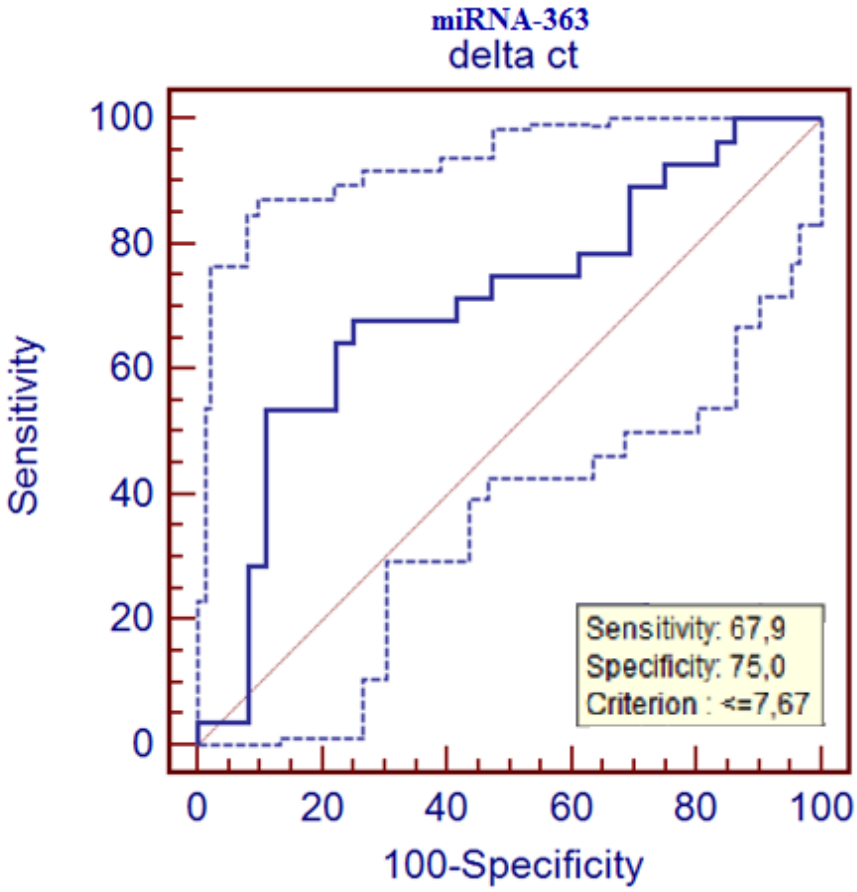
Positive group	
Diagnosisi	= 1
Sample size	28
Negative group	
Diagnosisi	= 0
Sample size	36

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,723
Standard Error	0,0629
95% Confidence Interval	0,597 to 0,828
z statistic	3,549
Significance level P (Area=0.5)	0,0004

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
< 18,89	0,00	0,0 - 12,5	100,00	90,2 - 100,0			1,00	
<=18,89	3,57	0,6 - 18,4	100,00	90,2 - 100,0			0,96	
<=19,46	7,14	1,1 - 23,5	100,00	90,2 - 100,0			0,93	
<=19,82	10,71	2,4 - 28,3	100,00	90,2 - 100,0			0,89	
<=19,93	10,71	2,4 - 28,3	97,22	85,4 - 99,5	3,86	1,3 - 11,3	0,92	0,1 - 6,4
<=19,98	10,71	2,4 - 28,3	94,44	81,3 - 99,2	1,93	0,7 - 5,6	0,95	0,2 - 3,7
<=20,29	14,29	4,1 - 32,7	94,44	81,3 - 99,2	2,57	1,0 - 6,4	0,91	0,2 - 3,5
<=20,37	17,86	6,1 - 36,9	94,44	81,3 - 99,2	3,21	1,4 - 7,1	0,87	0,2 - 3,4
<=20,63	17,86	6,1 - 36,9	91,67	77,5 - 98,2	2,14	1,0 - 4,8	0,90	0,3 - 2,7
<=20,67	21,43	8,3 - 41,0	91,67	77,5 - 98,2	2,57	1,3 - 5,3	0,86	0,3 - 2,6
<=21,2	25,00	10,7 - 44,9	91,67	77,5 - 98,2	3,00	1,6 - 5,7	0,82	0,3 - 2,5
<=21,46	25,00	10,7 - 44,9	88,89	73,9 - 96,8	2,25	1,2 - 4,3	0,84	0,3 - 2,2
<=21,65	28,57	13,3 - 48,7	88,89	73,9 - 96,8	2,57	1,4 - 4,7	0,80	0,3 - 2,1
<=21,67	32,14	15,9 - 52,3	88,89	73,9 - 96,8	2,89	1,7 - 5,0	0,76	0,3 - 2,0
<=21,68	35,71	18,7 - 55,9	88,89	73,9 - 96,8	3,21	1,9 - 5,4	0,72	0,3 - 1,9
<=22,78	39,29	21,5 - 59,4	88,89	73,9 - 96,8	3,54	2,2 - 5,7	0,68	0,3 - 1,8
<=22,84	42,86	24,5 - 62,8	88,89	73,9 - 96,8	3,86	2,5 - 6,0	0,64	0,2 - 1,7
<=23,48	46,43	27,5 - 66,1	88,89	73,9 - 96,8	4,18	2,8 - 6,3	0,60	0,2 - 1,6
<=24,01	50,00	30,7 - 69,3	88,89	73,9 - 96,8	4,50	3,1 - 6,6	0,56	0,2 - 1,5
<=24,26	53,57	33,9 - 72,5	88,89	73,9 - 96,8	4,82	3,4 - 6,9	0,52	0,2 - 1,4
<=24,57	57,14	37,2 - 75,5	88,89	73,9 - 96,8	5,14	3,7 - 7,2	0,48	0,2 - 1,3
<=24,72	57,14	37,2 - 75,5	86,11	70,5 - 95,3	4,11	2,9 - 5,8	0,50	0,2 - 1,2
<=24,83	60,71	40,6 - 78,5	86,11	70,5 - 95,3	4,37	3,2 - 6,1	0,46	0,2 - 1,2
<=25,08 *	64,29	44,1 - 81,3	86,11	70,5 - 95,3	4,63	3,4 - 6,3	0,41	0,2 - 1,1
<=25,26	64,29	44,1 - 81,3	83,33	67,2 - 93,6	3,86	2,8 - 5,3	0,43	0,2 - 1,0
<=25,33	64,29	44,1 - 81,3	80,56	64,0 - 91,8	3,31	2,4 - 4,5	0,44	0,2 - 1,0
<=25,74	64,29	44,1 - 81,3	77,78	60,8 - 89,9	2,89	2,1 - 4,0	0,46	0,2 - 1,0
<=26,36	67,86	47,7 - 84,1	77,78	60,8 - 89,9	3,05	2,2 - 4,2	0,41	0,2 - 0,9
<=26,7	67,86	47,7 - 84,1	75,00	57,8 - 87,9	2,71	2,0 - 3,7	0,43	0,2 - 0,9
<=26,88	67,86	47,7 - 84,1	72,22	54,8 - 85,8	2,44	1,8 - 3,4	0,45	0,2 - 0,9
<=27,01	67,86	47,7 - 84,1	69,44	51,9 - 83,6	2,22	1,6 - 3,1	0,46	0,2 - 1,0
<=27,29	67,86	47,7 - 84,1	66,67	49,0 - 81,4	2,04	1,4 - 2,9	0,48	0,2 - 1,0
<=28,13	67,86	47,7 - 84,1	63,89	46,2 - 79,2	1,88	1,3 - 2,7	0,50	0,3 - 1,0
<=28,66	67,86	47,7 - 84,1	61,11	43,5 - 76,8	1,74	1,2 - 2,5	0,53	0,3 - 1,0
<=28,96	67,86	47,7 - 84,1	58,33	40,8 - 74,5	1,63	1,1 - 2,4	0,55	0,3 - 1,1
<=29,13	67,86	47,7 - 84,1	55,56	38,1 - 72,1	1,53	1,0 - 2,2	0,58	0,3 - 1,1
<=29,43	71,43	51,3 - 86,7	55,56	38,1 - 72,1	1,61	1,1 - 2,3	0,51	0,3 - 1,0
<=29,88	71,43	51,3 - 86,7	52,78	35,5 - 69,6	1,51	1,0 - 2,2	0,54	0,3 - 1,1
<=30,19	75,00	55,1 - 89,3	52,78	35,5 - 69,6	1,59	1,1 - 2,3	0,47	0,2 - 1,0
<=30,83	75,00	55,1 - 89,3	50,00	32,9 - 67,1	1,50	1,0 - 2,2	0,50	0,2 - 1,0
<=31,05	75,00	55,1 - 89,3	47,22	30,4 - 64,5	1,42	0,9 - 2,1	0,53	0,3 - 1,1
<=31,56	75,00	55,1 - 89,3	44,44	27,9 - 61,9	1,35	0,9 - 2,1	0,56	0,3 - 1,1
<=32,13	75,00	55,1 - 89,3	41,67	25,5 - 59,2	1,29	0,8 - 2,0	0,60	0,3 - 1,2
<=32,49	75,00	55,1 - 89,3	38,89	23,2 - 56,5	1,23	0,8 - 1,9	0,64	0,3 - 1,3
<=33,26	75,00	55,1 - 89,3	36,11	20,8 - 53,8	1,17	0,7 - 1,9	0,69	0,3 - 1,4
<=34,33	78,57	59,0 - 91,7	36,11	20,8 - 53,8	1,23	0,8 - 2,0	0,59	0,3 - 1,3
<=34,77	82,14	63,1 - 93,9	36,11	20,8 - 53,8	1,29	0,8 - 2,1	0,49	0,2 - 1,1
<=34,87	82,14	63,1 - 93,9	33,33	18,6 - 51,0	1,23	0,8 - 2,0	0,54	0,2 - 1,2
<=35,08	82,14	63,1 - 93,9	30,56	16,4 - 48,1	1,18	0,7 - 2,0	0,58	0,3 - 1,3
<=35,74	82,14	63,1 - 93,9	27,78	14,2 - 45,2	1,14	0,7 - 2,0	0,64	0,3 - 1,5
<=36,07	85,71	67,3 - 95,9	27,78	14,2 - 45,2	1,19	0,7 - 2,1	0,51	0,2 - 1,3
<=36,6	89,29	71,7 - 97,6	27,78	14,2 - 45,2	1,24	0,7 - 2,1	0,39	0,1 - 1,1
<=37,12	89,29	71,7 - 97,6	25,00	12,1 - 42,2	1,19	0,7 - 2,1	0,43	0,1 - 1,3
<=38,18	89,29	71,7 - 97,6	22,22	10,1 - 39,2	1,15	0,6 - 2,1	0,48	0,2 - 1,4
<=38,44	92,86	76,5 - 98,9	22,22	10,1 - 39,2	1,19	0,6 - 2,2	0,32	0,08 - 1,2
<=39,1	96,43	81,6 - 99,4	22,22	10,1 - 39,2	1,24	0,7 - 2,3	0,16	0,02 - 1,1
<=39,15	100,00	87,5 - 100,0	22,22	10,1 - 39,2	1,29	0,7 - 2,4	0,00	
<=39,51	100,00	87,5 - 100,0	19,44	8,2 - 36,0	1,24	0,6 - 2,4	0,00	
<=40,38	100,00	87,5 - 100,0	16,67	6,4 - 32,8	1,20	0,6 - 2,5	0,00	
<=40,42	100,00	87,5 - 100,0	13,89	4,7 - 29,5	1,16	0,5 - 2,6	0,00	
<=41,78	100,00	87,5 - 100,0	11,11	3,2 - 26,1	1,12	0,4 - 2,8	0,00	
<=42,38	100,00	87,5 - 100,0	8,33	1,8 - 22,5	1,09	0,4 - 3,2	0,00	
<=42,9	100,00	87,5 - 100,0	5,56	0,8 - 18,7	1,06	0,3 - 4,1	0,00	
<=43,13	100,00	87,5 - 100,0	2,78	0,5 - 14,6	1,03	0,1 - 7,1	0,00	
<=44,07	100,00	87,5 - 100,0	0,00	0,0 - 9,8	1,00			



ROC curve

Variable	miRNA-363_delta_ct delta ct
Classification variable	Diagnosis

Positive group	
Diagnosis	= 1
Sample size	28
Negative group	
Diagnosis	= 0
Sample size	36

Disease prevalence (%)	43,7

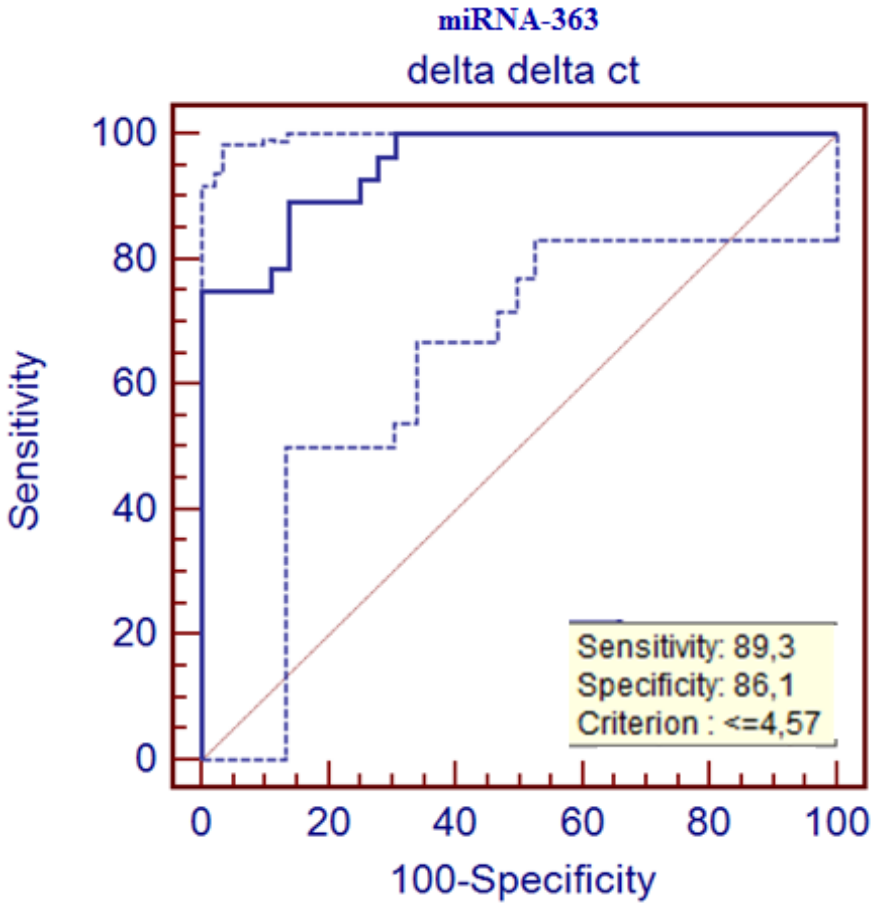
Area under the ROC curve (AUC)	0,703
Standard Error	0,0646
95% Confidence Interval	0,576 to 0,811
z statistic	3,149
Significance level P (Area=0.5)	0,0016

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -1,14	0,00	0,0 - 12,5	100,00	90,2 - 100,0			1,00				56,2	43,3 - 68,6
<=-1,14	3,57	0,6 - 18,4	100,00	90,2 - 100,0			0,96		100,0	16,5 - 100,0	57,1	44,0 - 69,5
<=-0,98	3,57	0,6 - 18,4	97,22	85,4 - 99,5	1,29	0,2 - 8,8	0,99	0,1 - 6,9	50,0	8,2 - 91,8	56,5	43,3 - 69,0
<=0,15	3,57	0,6 - 18,4	94,44	81,3 - 99,2	0,64	0,09 - 4,4	1,02	0,3 - 3,9	33,3	5,5 - 88,4	55,7	42,4 - 68,5
<=0,16	3,57	0,6 - 18,4	91,67	77,5 - 98,2	0,43	0,06 - 2,9	1,05	0,4 - 3,1	25,0	4,1 - 79,7	55,0	41,6 - 67,9
<=0,23	7,14	1,1 - 23,5	91,67	77,5 - 98,2	0,86	0,2 - 3,3	1,01	0,3 - 3,0	40,0	6,5 - 84,6	55,9	42,4 - 68,8

<=2,56	10,71	2,4 - 28,3	91,67	77,5 - 98,2	1,29	0,4 - 3,8	0,97	0,3 - 2,9	50,0	12,4 - 87,6	56,9	43,2 - 69,8
<=2,58	14,29	4,1 - 32,7	91,67	77,5 - 98,2	1,71	0,7 - 4,3	0,94	0,3 - 2,8	57,1	18,8 - 89,6	57,9	44,1 - 70,9
<=2,66	17,86	6,1 - 36,9	91,67	77,5 - 98,2	2,14	1,0 - 4,8	0,90	0,3 - 2,7	62,5	24,7 - 91,0	58,9	45,0 - 71,9
<=2,72	21,43	8,3 - 41,0	91,67	77,5 - 98,2	2,57	1,3 - 5,3	0,86	0,3 - 2,6	66,7	30,1 - 92,1	60,0	45,9 - 73,0
<=2,86	25,00	10,7 - 44,9	91,67	77,5 - 98,2	3,00	1,6 - 5,7	0,82	0,3 - 2,5	70,0	34,8 - 93,0	61,1	46,9 - 74,1
<=2,96	28,57	13,3 - 48,7	91,67	77,5 - 98,2	3,43	1,9 - 6,2	0,78	0,3 - 2,4	72,7	39,1 - 93,7	62,3	47,9 - 75,2
<=2,99	28,57	13,3 - 48,7	88,89	73,9 - 96,8	2,57	1,4 - 4,7	0,80	0,3 - 2,1	66,7	34,9 - 89,9	61,5	47,0 - 74,7
<=3,42	32,14	15,9 - 52,3	88,89	73,9 - 96,8	2,89	1,7 - 5,0	0,76	0,3 - 2,0	69,2	38,6 - 90,7	62,7	48,1 - 75,9
<=4,16	35,71	18,7 - 55,9	88,89	73,9 - 96,8	3,21	1,9 - 5,4	0,72	0,3 - 1,9	71,4	41,9 - 91,4	64,0	49,2 - 77,1
<=4,37	39,29	21,5 - 59,4	88,89	73,9 - 96,8	3,54	2,2 - 5,7	0,68	0,3 - 1,8	73,3	44,9 - 92,0	65,3	50,4 - 78,3
<=4,55	42,86	24,5 - 62,8	88,89	73,9 - 96,8	3,86	2,5 - 6,0	0,64	0,2 - 1,7	75,0	47,6 - 92,6	66,7	51,6 - 79,6
<=4,66	46,43	27,5 - 66,1	88,89	73,9 - 96,8	4,18	2,8 - 6,3	0,60	0,2 - 1,6	76,5	50,1 - 93,0	68,1	52,9 - 80,9
<=5,08	50,00	30,7 - 69,3	88,89	73,9 - 96,8	4,50	3,1 - 6,6	0,56	0,2 - 1,5	77,8	52,4 - 93,5	69,6	54,2 - 82,2
<=5,2	53,57	33,9 - 72,5	88,89	73,9 - 96,8	4,82	3,4 - 6,9	0,52	0,2 - 1,4	78,9	54,4 - 93,8	71,1	55,7 - 83,6
<=5,45	53,57	33,9 - 72,5	86,11	70,5 - 95,3	3,86	2,7 - 5,6	0,54	0,2 - 1,3	75,0	50,9 - 91,2	70,5	54,8 - 83,2
<=6,08	53,57	33,9 - 72,5	83,33	67,2 - 93,6	3,21	2,2 - 4,7	0,56	0,2 - 1,3	71,4	47,8 - 88,6	69,8	53,9 - 82,8
<=6,47	53,57	33,9 - 72,5	80,56	64,0 - 91,8	2,76	1,9 - 4,0	0,58	0,3 - 1,3	68,2	45,1 - 86,1	69,0	52,9 - 82,4
<=6,49	53,57	33,9 - 72,5	77,78	60,8 - 89,9	2,41	1,6 - 3,5	0,60	0,3 - 1,2	65,2	42,7 - 83,6	68,3	51,9 - 81,9
<=6,55	57,14	37,2 - 75,5	77,78	60,8 - 89,9	2,57	1,8 - 3,7	0,55	0,3 - 1,2	66,7	44,7 - 84,3	70,0	53,5 - 83,4
<=6,63	60,71	40,6 - 78,5	77,78	60,8 - 89,9	2,73	1,9 - 3,9	0,51	0,2 - 1,1	68,0	46,5 - 85,0	71,8	55,1 - 85,0
<=7,15	64,29	44,1 - 81,3	77,78	60,8 - 89,9	2,89	2,1 - 4,0	0,46	0,2 - 1,0	69,2	48,2 - 85,6	73,7	56,9 - 86,6
<=7,52	64,29	44,1 - 81,3	75,00	57,8 - 87,9	2,57	1,8 - 3,6	0,48	0,2 - 1,0	66,7	46,0 - 83,4	73,0	55,9 - 86,2
<=7,67 *	67,86	47,7 - 84,1	75,00	57,8 - 87,9	2,71	2,0 - 3,7	0,43	0,2 - 0,9	67,9	47,7 - 84,1	75,0	57,8 - 87,9
<=7,84	67,86	47,7 - 84,1	72,22	54,8 - 85,8	2,44	1,8 - 3,4	0,45	0,2 - 0,9	65,5	45,7 - 82,0	74,3	56,7 - 87,5
<=8,13	67,86	47,7 - 84,1	69,44	51,9 - 83,6	2,22	1,6 - 3,1	0,46	0,2 - 1,0	63,3	43,9 - 80,0	73,5	55,6 - 87,1
<=8,48	67,86	47,7 - 84,1	66,67	49,0 - 81,4	2,04	1,4 - 2,9	0,48	0,2 - 1,0	61,3	42,2 - 78,1	72,7	54,5 - 86,7
<=8,54	67,86	47,7 - 84,1	63,89	46,2 - 79,2	1,88	1,3 - 2,7	0,50	0,3 - 1,0	59,4	40,7 - 76,3	71,9	53,3 - 86,2
<=8,89	67,86	47,7 - 84,1	61,11	43,5 - 76,8	1,74	1,2 - 2,5	0,53	0,3 - 1,0	57,6	39,2 - 74,5	71,0	52,0 - 85,7
<=9,39	67,86	47,7 - 84,1	58,33	40,8 - 74,5	1,63	1,1 - 2,4	0,55	0,3 - 1,1	55,9	37,9 - 72,8	70,0	50,6 - 85,2
<=10,42	71,43	51,3 - 86,7	58,33	40,8 - 74,5	1,71	1,2 - 2,5	0,49	0,2 - 1,0	57,1	39,4 - 73,7	72,4	52,8 - 87,2
<=10,48	71,43	51,3 - 86,7	55,56	38,1 - 72,1	1,61	1,1 - 2,3	0,51	0,3 - 1,0	55,6	38,1 - 72,1	71,4	51,3 - 86,7
<=10,54	71,43	51,3 - 86,7	52,78	35,5 - 69,6	1,51	1,0 - 2,2	0,54	0,3 - 1,1	54,1	36,9 - 70,5	70,4	49,8 - 86,2
<=10,6	75,00	55,1 - 89,3	52,78	35,5 - 69,6	1,59	1,1 - 2,3	0,47	0,2 - 1,0	55,3	38,3 - 71,4	73,1	52,2 - 88,4
<=11,07	75,00	55,1 - 89,3	50,00	32,9 - 67,1	1,50	1,0 - 2,2	0,50	0,2 - 1,0	53,8	37,2 - 69,9	72,0	50,6 - 87,9
<=12,04	75,00	55,1 - 89,3	47,22	30,4 - 64,5	1,42	0,9 - 2,1	0,53	0,3 - 1,1	52,5	36,1 - 68,5	70,8	48,9 - 87,3
<=12,66	75,00	55,1 - 89,3	44,44	27,9 - 61,9	1,35	0,9 - 2,1	0,56	0,3 - 1,1	51,2	35,1 - 67,1	69,6	47,1 - 86,7
<=13,13	75,00	55,1 - 89,3	41,67	25,5 - 59,2	1,29	0,8 - 2,0	0,60	0,3 - 1,2	50,0	34,2 - 65,8	68,2	45,1 - 86,1
<=13,62	75,00	55,1 - 89,3	38,89	23,2 - 56,5	1,23	0,8 - 1,9	0,64	0,3 - 1,3	48,8	33,3 - 64,5	66,7	43,0 - 85,4
<=14,86	78,57	59,0 - 91,7	38,89	23,2 - 56,5	1,29	0,8 - 2,0	0,55	0,3 - 1,2	50,0	34,6 - 65,4	70,0	45,7 - 88,0

<=14,97	78,57	59,0 - 91,7	36,11	20,8 - 53,8	1,23	0,8 - 2,0	0,59	0,3 - 1,3	48,9	33,7 - 64,2	68,4	43,5 - 87,3
<=15,15	78,57	59,0 - 91,7	33,33	18,6 - 51,0	1,18	0,7 - 1,9	0,64	0,3 - 1,4	47,8	32,9 - 63,1	66,7	41,0 - 86,6
<=15,3	78,57	59,0 - 91,7	30,56	16,4 - 48,1	1,13	0,7 - 1,9	0,70	0,3 - 1,5	46,8	32,1 - 61,9	64,7	38,4 - 85,7
<=15,46	82,14	63,1 - 93,9	30,56	16,4 - 48,1	1,18	0,7 - 2,0	0,58	0,3 - 1,3	47,9	33,3 - 62,8	68,7	41,4 - 88,9
<=16,1	85,71	67,3 - 95,9	30,56	16,4 - 48,1	1,23	0,7 - 2,1	0,47	0,2 - 1,2	49,0	34,4 - 63,7	73,3	44,9 - 92,0
<=17,02	89,29	71,7 - 97,6	30,56	16,4 - 48,1	1,29	0,8 - 2,1	0,35	0,1 - 1,0	50,0	35,5 - 64,5	78,6	49,2 - 95,1
<=17,99	89,29	71,7 - 97,6	27,78	14,2 - 45,2	1,24	0,7 - 2,1	0,39	0,1 - 1,1	49,0	34,8 - 63,4	76,9	46,2 - 94,7
<=18,47	89,29	71,7 - 97,6	25,00	12,1 - 42,2	1,19	0,7 - 2,1	0,43	0,1 - 1,3	48,1	34,0 - 62,4	75,0	42,8 - 94,2
<=19,75	92,86	76,5 - 98,9	25,00	12,1 - 42,2	1,24	0,7 - 2,2	0,29	0,07 - 1,1	49,1	35,1 - 63,2	81,8	48,2 - 97,2
<=19,98	92,86	76,5 - 98,9	22,22	10,1 - 39,2	1,19	0,6 - 2,2	0,32	0,08 - 1,2	48,1	34,3 - 62,2	80,0	44,4 - 96,9
<=20,06	92,86	76,5 - 98,9	19,44	8,2 - 36,0	1,15	0,6 - 2,3	0,37	0,1 - 1,4	47,3	33,7 - 61,2	77,8	40,1 - 96,5
<=20,17	92,86	76,5 - 98,9	16,67	6,4 - 32,8	1,11	0,5 - 2,3	0,43	0,1 - 1,6	46,4	33,0 - 60,3	75,0	35,0 - 96,1
<=20,26	96,43	81,6 - 99,4	16,67	6,4 - 32,8	1,16	0,6 - 2,4	0,21	0,03 - 1,5	47,4	34,0 - 61,0	85,7	42,2 - 97,6
<=20,34	96,43	81,6 - 99,4	13,89	4,7 - 29,5	1,12	0,5 - 2,5	0,26	0,04 - 1,8	46,6	33,3 - 60,1	83,3	36,1 - 97,2
<=20,39	100,00	87,5 - 100,0	13,89	4,7 - 29,5	1,16	0,5 - 2,6	0,00		47,5	34,3 - 60,9	100,0	48,0 - 100,0
<=22,38	100,00	87,5 - 100,0	11,11	3,2 - 26,1	1,12	0,4 - 2,8	0,00		46,7	33,7 - 60,0	100,0	40,2 - 100,0
<=22,42	100,00	87,5 - 100,0	8,33	1,8 - 22,5	1,09	0,4 - 3,2	0,00		45,9	33,1 - 59,1	100,0	30,5 - 100,0
<=22,96	100,00	87,5 - 100,0	5,56	0,8 - 18,7	1,06	0,3 - 4,1	0,00		45,2	32,5 - 58,3	100,0	19,3 - 100,0
<=23,74	100,00	87,5 - 100,0	2,78	0,5 - 14,6	1,03	0,1 - 7,1	0,00		44,4	31,9 - 57,5	100,0	16,5 - 100,0
<=25,08	100,00	87,5 - 100,0	0,00	0,0 - 9,8	1,00				43,7	31,4 - 56,7		



ROC curve

Variable	miRNA-363_delta_delta_ct delta delta ct
Classification variable	diagnosis

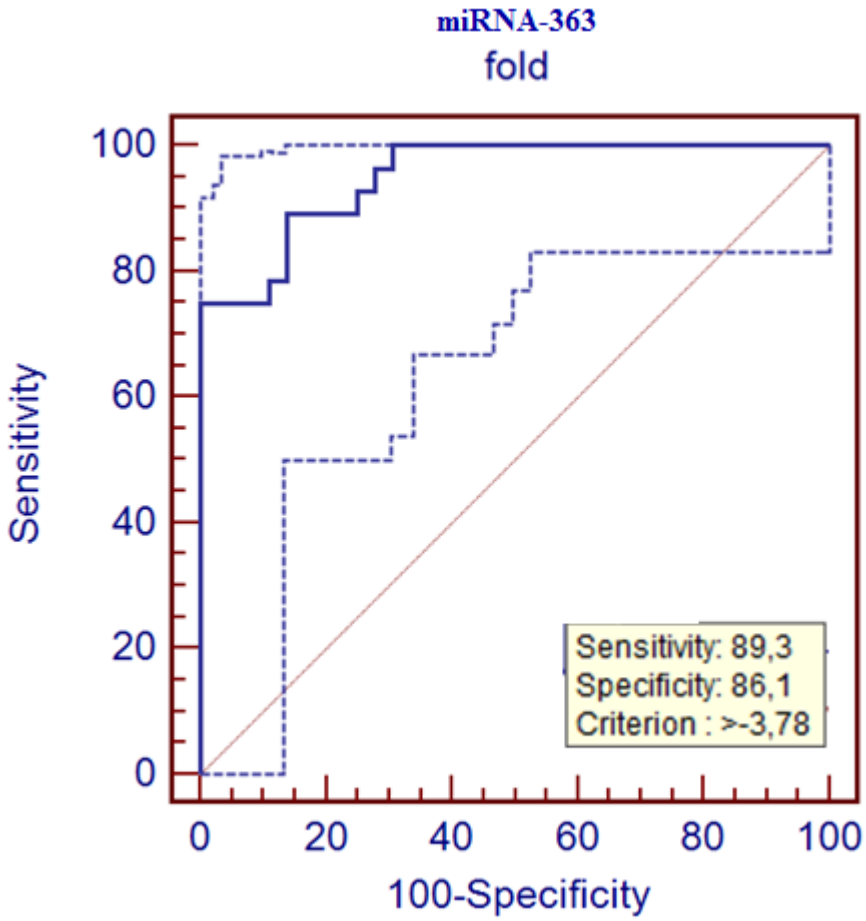
Positive group	
diagnosis	= 1
Sample size	28
Negative group	
diagnosis	= 0
Sample size	36

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,951
Standard Error	0,0269
95% Confidence Interval	0,866 to 0,989
z statistic	16,772
Significance level P (Area=0.5)	0,0001

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
< -13,59	0,00	0,0 - 12,5	100,00	90,2 - 100,0			1,00	
<=-13,59	3,57	0,6 - 18,4	100,00	90,2 - 100,0			0,96	
<=-12,22	7,14	1,1 - 23,5	100,00	90,2 - 100,0			0,93	
<=-9,89	10,71	2,4 - 28,3	100,00	90,2 - 100,0			0,89	
<=-9,87	14,29	4,1 - 32,7	100,00	90,2 - 100,0			0,86	
<=-9,79	17,86	6,1 - 36,9	100,00	90,2 - 100,0			0,82	
<=-9,73	21,43	8,3 - 41,0	100,00	90,2 - 100,0			0,79	
<=-9,59	25,00	10,7 - 44,9	100,00	90,2 - 100,0			0,75	
<=-9,49	28,57	13,3 - 48,7	100,00	90,2 - 100,0			0,71	
<=-9,03	32,14	15,9 - 52,3	100,00	90,2 - 100,0			0,68	
<=-8,29	35,71	18,7 - 55,9	100,00	90,2 - 100,0			0,64	
<=-8,08	39,29	21,5 - 59,4	100,00	90,2 - 100,0			0,61	
<=-7,9	42,86	24,5 - 62,8	100,00	90,2 - 100,0			0,57	
<=-7,79	46,43	27,5 - 66,1	100,00	90,2 - 100,0			0,54	
<=-7,37	50,00	30,7 - 69,3	100,00	90,2 - 100,0			0,50	
<=-7,25	53,57	33,9 - 72,5	100,00	90,2 - 100,0			0,46	
<=-5,9	57,14	37,2 - 75,5	100,00	90,2 - 100,0			0,43	
<=-5,82	60,71	40,6 - 78,5	100,00	90,2 - 100,0			0,39	
<=-5,3	64,29	44,1 - 81,3	100,00	90,2 - 100,0			0,36	
<=-4,78	67,86	47,7 - 84,1	100,00	90,2 - 100,0			0,32	
<=-2,03	71,43	51,3 - 86,7	100,00	90,2 - 100,0			0,29	
<=-1,85	75,00	55,1 - 89,3	100,00	90,2 - 100,0			0,25	
<=-0,98	75,00	55,1 - 89,3	97,22	85,4 - 99,5	27,00	21,6 - 33,7	0,26	0,03 - 2,0
<=0,15	75,00	55,1 - 89,3	94,44	81,3 - 99,2	13,50	10,7 - 17,0	0,26	0,06 - 1,2
<=0,16	75,00	55,1 - 89,3	91,67	77,5 - 98,2	9,00	7,1 - 11,4	0,27	0,08 - 1,0
<=0,68	75,00	55,1 - 89,3	88,89	73,9 - 96,8	6,75	5,3 - 8,6	0,28	0,09 - 0,9
<=2,41	78,57	59,0 - 91,7	88,89	73,9 - 96,8	7,07	5,6 - 8,9	0,24	0,08 - 0,8
<=2,99	78,57	59,0 - 91,7	86,11	70,5 - 95,3	5,66	4,5 - 7,1	0,25	0,08 - 0,7
<=3,01	82,14	63,1 - 93,9	86,11	70,5 - 95,3	5,91	4,8 - 7,3	0,21	0,07 - 0,6
<=3,65	85,71	67,3 - 95,9	86,11	70,5 - 95,3	6,17	5,1 - 7,5	0,17	0,05 - 0,6
<=4,57 *	89,29	71,7 - 97,6	86,11	70,5 - 95,3	6,43	5,4 - 7,7	0,12	0,03 - 0,5
<=5,45	89,29	71,7 - 97,6	83,33	67,2 - 93,6	5,36	4,4 - 6,5	0,13	0,04 - 0,5
<=6,08	89,29	71,7 - 97,6	80,56	64,0 - 91,8	4,59	3,7 - 5,6	0,13	0,04 - 0,5
<=6,47	89,29	71,7 - 97,6	77,78	60,8 - 89,9	4,02	3,2 - 5,0	0,14	0,04 - 0,5
<=6,49	89,29	71,7 - 97,6	75,00	57,8 - 87,9	3,57	2,8 - 4,5	0,14	0,04 - 0,5
<=7,3	92,86	76,5 - 98,9	75,00	57,8 - 87,9	3,71	3,0 - 4,6	0,095	0,02 - 0,4
<=7,52	92,86	76,5 - 98,9	72,22	54,8 - 85,8	3,34	2,7 - 4,2	0,099	0,02 - 0,4
<=7,81	96,43	81,6 - 99,4	72,22	54,8 - 85,8	3,47	2,8 - 4,3	0,049	0,007 - 0,4
<=7,84	96,43	81,6 - 99,4	69,44	51,9 - 83,6	3,16	2,5 - 4,0	0,051	0,007 - 0,4
<=7,94	100,00	87,5 - 100,0	69,44	51,9 - 83,6	3,27	2,6 - 4,1	0,00	
<=8,13	100,00	87,5 - 100,0	66,67	49,0 - 81,4	3,00	2,4 - 3,8	0,00	
<=8,48	100,00	87,5 - 100,0	63,89	46,2 - 79,2	2,77	2,2 - 3,5	0,00	
<=8,54	100,00	87,5 - 100,0	61,11	43,5 - 76,8	2,57	2,0 - 3,3	0,00	
<=8,89	100,00	87,5 - 100,0	58,33	40,8 - 74,5	2,40	1,8 - 3,2	0,00	
<=9,39	100,00	87,5 - 100,0	55,56	38,1 - 72,1	2,25	1,7 - 3,0	0,00	
<=10,48	100,00	87,5 - 100,0	52,78	35,5 - 69,6	2,12	1,6 - 2,9	0,00	
<=10,54	100,00	87,5 - 100,0	50,00	32,9 - 67,1	2,00	1,4 - 2,8	0,00	
<=11,07	100,00	87,5 - 100,0	47,22	30,4 - 64,5	1,89	1,3 - 2,7	0,00	
<=12,04	100,00	87,5 - 100,0	44,44	27,9 - 61,9	1,80	1,2 - 2,6	0,00	
<=12,66	100,00	87,5 - 100,0	41,67	25,5 - 59,2	1,71	1,2 - 2,5	0,00	
<=13,62	100,00	87,5 - 100,0	38,89	23,2 - 56,5	1,64	1,1 - 2,5	0,00	
<=14,97	100,00	87,5 - 100,0	36,11	20,8 - 53,8	1,57	1,0 - 2,4	0,00	
<=15,15	100,00	87,5 - 100,0	33,33	18,6 - 51,0	1,50	0,9 - 2,4	0,00	
<=15,3	100,00	87,5 - 100,0	30,56	16,4 - 48,1	1,44	0,9 - 2,4	0,00	
<=17,99	100,00	87,5 - 100,0	27,78	14,2 - 45,2	1,38	0,8 - 2,3	0,00	
<=18,47	100,00	87,5 - 100,0	25,00	12,1 - 42,2	1,33	0,8 - 2,3	0,00	
<=19,98	100,00	87,5 - 100,0	22,22	10,1 - 39,2	1,29	0,7 - 2,4	0,00	
<=20,06	100,00	87,5 - 100,0	19,44	8,2 - 36,0	1,24	0,6 - 2,4	0,00	
<=20,17	100,00	87,5 - 100,0	16,67	6,4 - 32,8	1,20	0,6 - 2,5	0,00	
<=20,34	100,00	87,5 - 100,0	13,89	4,7 - 29,5	1,16	0,5 - 2,6	0,00	
<=22,38	100,00	87,5 - 100,0	11,11	3,2 - 26,1	1,12	0,4 - 2,8	0,00	
<=22,42	100,00	87,5 - 100,0	8,33	1,8 - 22,5	1,09	0,4 - 3,2	0,00	
<=22,96	100,00	87,5 - 100,0	5,56	0,8 - 18,7	1,06	0,3 - 4,1	0,00	
<=23,74	100,00	87,5 - 100,0	2,78	0,5 - 14,6	1,03	0,1 - 7,1	0,00	
<=25,08	100,00	87,5 - 100,0	0,00	0,0 - 9,8	1,00			



ROC curve

Variable	miRNA-363_fold	change
Classification variable	diagnosis	

Positive group	
diagnosis	= 1
Sample size	28
Negative group	
diagnosis	= 0
Sample size	36

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)	0,951
Standard Error	0,0298
95% Confidence Interval	0,866 to 0,989
z statistic	15,163
Significance level P (Area=0.5)	0,0001

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
>=-17,38	100,00	87,5 - 100,0	0,00	0,0 - 9,8	1,00			
>-17,38	100,00	87,5 - 100,0	2,78	0,5 - 14,6	1,03	0,1 - 7,1	0,00	
>-16,46	100,00	87,5 - 100,0	5,56	0,8 - 18,7	1,06	0,3 - 4,1	0,00	
>-15,91	100,00	87,5 - 100,0	8,33	1,8 - 22,5	1,09	0,4 - 3,2	0,00	
>-15,54	100,00	87,5 - 100,0	11,11	3,2 - 26,1	1,12	0,4 - 2,8	0,00	
>-15,51	100,00	87,5 - 100,0	13,89	4,7 - 29,5	1,16	0,5 - 2,6	0,00	
>-14,1	100,00	87,5 - 100,0	16,67	6,4 - 32,8	1,20	0,6 - 2,5	0,00	
>-13,98	100,00	87,5 - 100,0	19,44	8,2 - 36,0	1,24	0,6 - 2,4	0,00	
>-13,9	100,00	87,5 - 100,0	22,22	10,1 - 39,2	1,29	0,7 - 2,4	0,00	
>-13,85	100,00	87,5 - 100,0	25,00	12,1 - 42,2	1,33	0,8 - 2,3	0,00	
>-12,8	100,00	87,5 - 100,0	27,78	14,2 - 45,2	1,38	0,8 - 2,3	0,00	
>-12,47	100,00	87,5 - 100,0	30,56	16,4 - 48,1	1,44	0,9 - 2,4	0,00	
>-10,61	100,00	87,5 - 100,0	33,33	18,6 - 51,0	1,50	0,9 - 2,4	0,00	
>-10,5	100,00	87,5 - 100,0	36,11	20,8 - 53,8	1,57	1,0 - 2,4	0,00	
>-10,38	100,00	87,5 - 100,0	38,89	23,2 - 56,5	1,64	1,1 - 2,5	0,00	
>-9,44	100,00	87,5 - 100,0	41,67	25,5 - 59,2	1,71	1,2 - 2,5	0,00	
>-8,78	100,00	87,5 - 100,0	44,44	27,9 - 61,9	1,80	1,2 - 2,6	0,00	
>-8,35	100,00	87,5 - 100,0	47,22	30,4 - 64,5	1,89	1,3 - 2,7	0,00	
>-7,67	100,00	87,5 - 100,0	50,00	32,9 - 67,1	2,00	1,4 - 2,8	0,00	
>-7,31	100,00	87,5 - 100,0	52,78	35,5 - 69,6	2,12	1,6 - 2,9	0,00	
>-7,26	100,00	87,5 - 100,0	55,56	38,1 - 72,1	2,25	1,7 - 3,0	0,00	
>-6,51	100,00	87,5 - 100,0	58,33	40,8 - 74,5	2,40	1,8 - 3,2	0,00	
>-6,16	100,00	87,5 - 100,0	61,11	43,5 - 76,8	2,57	2,0 - 3,3	0,00	
>-5,92	100,00	87,5 - 100,0	63,89	46,2 - 79,2	2,77	2,2 - 3,5	0,00	
>-5,88	100,00	87,5 - 100,0	66,67	49,0 - 81,4	3,00	2,4 - 3,8	0,00	
>-5,64	100,00	87,5 - 100,0	69,44	51,9 - 83,6	3,27	2,6 - 4,1	0,00	
>-5,5	96,43	81,6 - 99,4	69,44	51,9 - 83,6	3,16	2,5 - 4,0	0,051	0,007 - 0,4
>-5,43	96,43	81,6 - 99,4	72,22	54,8 - 85,8	3,47	2,8 - 4,3	0,049	0,007 - 0,4
>-5,41	92,86	76,5 - 98,9	72,22	54,8 - 85,8	3,34	2,7 - 4,2	0,099	0,02 - 0,4
>-5,21	92,86	76,5 - 98,9	75,00	57,8 - 87,9	3,71	3,0 - 4,6	0,095	0,02 - 0,4
>-5,06	89,29	71,7 - 97,6	75,00	57,8 - 87,9	3,57	2,8 - 4,5	0,14	0,04 - 0,5
>-4,5	89,29	71,7 - 97,6	77,78	60,8 - 89,9	4,02	3,2 - 5,0	0,14	0,04 - 0,5
>-4,48	89,29	71,7 - 97,6	80,56	64,0 - 91,8	4,59	3,7 - 5,6	0,13	0,04 - 0,5
>-4,21	89,29	71,7 - 97,6	83,33	67,2 - 93,6	5,36	4,4 - 6,5	0,13	0,04 - 0,5
>-3,78 *	89,29	71,7 - 97,6	86,11	70,5 - 95,3	6,43	5,4 - 7,7	0,12	0,03 - 0,5
>-3,17	85,71	67,3 - 95,9	86,11	70,5 - 95,3	6,17	5,1 - 7,5	0,17	0,05 - 0,6
>-2,53	82,14	63,1 - 93,9	86,11	70,5 - 95,3	5,91	4,8 - 7,3	0,21	0,07 - 0,6
>-2,09	78,57	59,0 - 91,7	86,11	70,5 - 95,3	5,66	4,5 - 7,1	0,25	0,08 - 0,7
>-2,07	78,57	59,0 - 91,7	88,89	73,9 - 96,8	7,07	5,6 - 8,9	0,24	0,08 - 0,8
>-1,67	75,00	55,1 - 89,3	88,89	73,9 - 96,8	6,75	5,3 - 8,6	0,28	0,09 - 0,9
>-0,47	75,00	55,1 - 89,3	91,67	77,5 - 98,2	9,00	7,1 - 11,4	0,27	0,08 - 1,0
>-0,11	75,00	55,1 - 89,3	94,44	81,3 - 99,2	13,50	10,7 - 17,0	0,26	0,06 - 1,2
>-0,1	75,00	55,1 - 89,3	97,22	85,4 - 99,5	27,00	21,6 - 33,7	0,26	0,03 - 2,0
>0,68	75,00	55,1 - 89,3	100,00	90,2 - 100,0			0,25	
>1,28	71,43	51,3 - 86,7	100,00	90,2 - 100,0			0,29	
>1,41	67,86	47,7 - 84,1	100,00	90,2 - 100,0			0,32	
>3,31	64,29	44,1 - 81,3	100,00	90,2 - 100,0			0,36	
>3,67	60,71	40,6 - 78,5	100,00	90,2 - 100,0			0,39	
>4,03	57,14	37,2 - 75,5	100,00	90,2 - 100,0			0,43	
>4,09	53,57	33,9 - 72,5	100,00	90,2 - 100,0			0,46	
>5,02	50,00	30,7 - 69,3	100,00	90,2 - 100,0			0,50	
>5,11	46,43	27,5 - 66,1	100,00	90,2 - 100,0			0,54	
>5,4	42,86	24,5 - 62,8	100,00	90,2 - 100,0			0,57	
>5,47	39,29	21,5 - 59,4	100,00	90,2 - 100,0			0,61	
>5,6	35,71	18,7 - 55,9	100,00	90,2 - 100,0			0,64	
>5,75	32,14	15,9 - 52,3	100,00	90,2 - 100,0			0,68	
>6,26	28,57	13,3 - 48,7	100,00	90,2 - 100,0			0,71	
>6,58	25,00	10,7 - 44,9	100,00	90,2 - 100,0			0,75	
>6,65	21,43	8,3 - 41,0	100,00	90,2 - 100,0			0,79	
>6,74	17,86	6,1 - 36,9	100,00	90,2 - 100,0			0,82	
>6,78	14,29	4,1 - 32,7	100,00	90,2 - 100,0			0,86	
>6,84	10,71	2,4 - 28,3	100,00	90,2 - 100,0			0,89	
>6,85	7,14	1,1 - 23,5	100,00	90,2 - 100,0			0,93	
>8,47	3,57	0,6 - 18,4	100,00	90,2 - 100,0			0,96	
>9,42	0,00	0,0 - 12,5	100,00	90,2 - 100,0			1,00	

FORMS

	YEDİTEPE ÜNİVERSİTESİ OLGU RAPOR FORMU

ÇALIŞMANI ADI: **Glioblastoma Multiforme Tümörlerinde miRNA 582-5p ve miRNA 363'ün Ekspresyon Düzeylerinin Kaspaz-9 Üzerine Etkisi**

ÇALIŞMAYA / ARAŞTIRMAYA DAHİL **EDİLME** KRİTERLERİ

Deney Grubu için ;

- Gönüllü Olma
- Glioblastoma Multiforme hastası olma
- 18 – 70 yaş aralığında olma
- Yukarıda belirtilenler haricinde bir hastalığa sahip olmama

Kontrol Grubu için ;

- Gönüllü olma
- Sağlıklı olma (yukarıda belirtilenlerde dahil olmak üzere hiçbir hastalığa sahip olmama)
- 18 – 70 yaş aralığında olma

ÇALIŞMAYA /ARAŞTIRMAYA DAHİL **EDİLMEME** KRİTERLERİ

- Gönüllü olmama
- 18 – 70 yaş aralığı dışında olma
- Yukarıda belirtilenler dışında bir hastalığa sahip olma

Sorumlu Araştırmacı

KLİNİK ARAŞTIRMALARDA KULLANILACAK BİYOLOJİK MATERYAL TRANSFER ANLAŞMASI

Araştırmanın Açık Adı: Glioblastoma Multiforme Tümörlerinde miRNA 582-5p ve miRNA 363'ün Ekspresyon Düzeylerinin Kaspaz-9 Üzerine Etkisi

Protokol Numarası :

Araştırmanın Özeti :
Primer malign beyin tümörleri 2 yıllık ortalama sağkalım süresi ile ölüme sebebiyet veren neoplaziler grubunda önemli yere sahiptir. Glioblastoma multiforme (GBM), santral sinir sisteminin en sık görülen ve en malign glial tümörüdür. GBM, malign astrositomların %80'ini oluşturur ve sağkalım süresi en kısa olan tümörlerden biridir. Standart tedavi ile tanı konulmasını takiben ortalama sağkalım süresi 14-16 aydır. Yapılan çalışmalar göstermiştir ki 3 yıldan fazla yaşam süresine sahip hastaların oranı tüm hastaların %2-3 kadarıdır.
MikroRNA (miRNA)'lar son yıllarda ortaya çıkan, 18-24 nükleotid uzunluğunda küçük kodlama yapmayan (noncoding) ve büyük bir çoğunluğu hücre içinde bulunmakla birlikte ekstrasellüler alanda, vücut sıvılarında ve kanda bulunabilen RNA moleküleridir. miRNA'lar hücre proliferasyonu, farklılaşması, metabolik süreçler, apoptoz, inflamasyon ve immünolojik süreçlerin düzenlenmesi gibi yolaklarda düzenleyicidirler. Çeşitli çalışmalarda yapılan gözlemler, insan plazmasında bulunan miRNA'ların yüksek bir stabiliteye sahip olduğunu ve bu örneklerde yapılan miRNA ekspresyon kalıbı analizinin çeşitli hastalıkların durumları hakkında yararlı bilgiler sunabileceğini ortaya koymuştur.
Son zamanlarda, miRNA'ların tümör büyümesi ve sağ kalımında kilit faktör olarak ortaya çıkması, normal beyin dokusuna kıyasla farklı tiplerdeki glioblastomlarda miRNA ekspresyon seviyelerinin değerlendirilmesine yol açmıştır. Sinir kök hücreleri (NSC) kullanılarak yapılan çalışmalar, nöral gelişimde yer alan miRNA'ların GBM gelişiminde rol oynadığını ve bilinen miRNA'ların % 70'inin beyinde ifade edildiğini göstermiştir.
Bu tez çalışmasının da hedef miRNA'ları olan miRNA 582-5p ve miRNA 363'ün onkojenik potansiyele sahip olduğu, GBM nörosfer kök hücre hatları üzerinde yapılan çalışmalarla gösterilmiştir. Ayrıca miRNA ile tedavi edilen hücre hatlarında apoptotik aktivitede bir azalma gözlemlenmiştir. Elde edilen bu sonuçlar, miRNA 582-5p ve miRNA 363'ün anti apoptotik etki mekanizmasını desteklemektedir.
Gerçekleştirilen biyoinformatik çalışmalar ve literatürden elde edilen bilgiler ışığında bu tez çalışması kapsamında; GBM tanısı almış hastalardan alınacak örnekler üzerinde, apoptotik etki mekanizması gösterilmiş olan miRNA 582-5p ve miRNA 363'ün ekspresyon seviyeleri belirlenerek, kontrol grubu ile ekspresyon düzeyi açısından bir fark olup olmadığı saptanarak araştırmaya konu olan miRNA'ların ekspresyon düzeylerinin kaspaz-9 aktivitesi üzerine olan etkisinin araştırılması hedeflenmiştir.

İşbu anlaşma ile biyolojik materyali gönderen araştırmacı ve kurum "**Glioblastoma multiforme tümörlerinde miRNA 582-5p ve miRNA 363'ün ekspresyon düzeylerinin kaspaz-9 üzerine etkisi** " isimli araştırmada kullanılmak üzere gönderilecek " **5 ml ve araştırma**" amacı ile kullanılması üzere **26 Ağustos Yerleşkesi Yeditepe Üniversitesi Kayışdağı / İSTANBUL** adresindeki **Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalı ' ndaki** merkezine göndermeden önce GÖNDERİCİ ve ALICI'dan aşağıdaki koşulları kabul etmesi istenmektedir;

AGREEMENT FOR TRANSFER OF BIOLOGICAL MATERIAL TO BE USED IN CLINICAL TRIALS

Full name of the clinical trial:

Protocol code :

Summary of the clinical trial:

Click here on enter text

By this agreement, the investigator and the institution who send the biological material requires the CONSIGNEE and CONSIGNOR to agree on the below terms before sending *(Specify biological material type and amount)* which shall be used for to be dispatched to the address of .

1. Delivered biological materials shall be used only for the above-mentioned purposes. CONSIGNEE shall use those materials only for secondary purposes, which are initially approved by the CONSIGNOR in written.
2. Prior to the dispatch of the biological materials to the CONSIGNEE, Turkish Medicines and Medical Devices Agency and Ethics Committee approved informed consent forms, which belong to the persons for whom the biological material is provided, should be obtained. This consent form should explain all the purposes of use of the biological samples.
3. CONSIGNEE cannot provide the biological material to the third parties without prior written approval of the CONSIGNOR.
4. Biological materials shall be dispatched by the CONSIGNOR to the CONSIGNEE without the identity or any descriptive information of the individuals.
5. CONSIGNEE shall use the biological materials in accordance with as the United Nations Human Genome and Universal Declaration of Human Rights.
6. CONSIGNEE acknowledges and agrees that the biological materials to be dispatched under this agreement shall be utilized for research purposes and have some risks associated with their usage. Appropriate preventive actions should be taken for those risks.
7. CONSIGNOR and CONSIGNEE shall mutually agree that the biological materials cannot be used as a source for any commercial profit and the rights relating to a joint publication or a patent right that may arise may be the only exception for that. CONSIGNOR and CONSIGNEE shall mutually agree on those rights prior to trial initiation.
8. CONSIGNEE agrees to return or dispose of all materials and to evidence such acts accordingly in the event of termination of the agreement or withdrawal of written consent of the volunteer referred in Item 2.
9. This agreement shall be terminated in the event of, termination of the trial, violation on the terms of related regulations or noncompliance with agreement clauses of either of the parties.
10. CONSIGNEE and CONSIGNOR shall be responsible from the execution of this Agreement and performances hereunder. In case of conflict, both countries of the parties' courts are authorized.

1. Gönderilen biyolojik materyaller yalnızca yukarıda yazılı amaç için kullanılabilir. ALICI biyolojik materyallerin alınma amacından dışında ikincil amaç için kullanılma isteklerini GÖNDERİCİ'ye yazılı olarak bildirecektir.
2. Biyolojik materyaller ALICI'ya gönderilmeden önce biyolojik materyalin sağlandığı gönüllülere ait Türkiye İlaç ve Tıbbi Cihaz Kurumu ve Etik Kurul'un onayladığı tüm kullanım amaçlarına yönelik olarak düzenlenmiş bilgilendirilmiş gönüllü olur formunun her bir gönüllüden alınmış olması gerekmektedir.
3. ALICI biyolojik materyali GÖNDERİCİ'nin yazılı izni olmadan üçüncü kişi/kurumlara vermeyecektir.
4. Biyolojik materyaller GÖNDERİCİ tarafından bireyin kimlik ve tanımlayıcı bilgileri olmaksızın ALICI'ya gönderilecektir.
5. ALICI biyolojik materyalleri Birleşmiş Milletler İnsan Genomu ve İnsan Hakları Evrensel Beyannamesine uygun olarak kullanacaktır.
6. Bu anlaşma ile gönderilecek biyolojik materyalin araştırma için kullanılacak olduğu ve biyolojik materyal kullanımına ait risklerin var olduğu ALICI tarafından kabul edilmektedir. Söz konusu risklere karşı uygun önlemlerin alınması gerekmektedir.
7. GÖNDERİCİ ve ALICI gönderilen biyolojik materyalin herhangi bir şekilde ticari kazanç kaynağı olarak kullanılamayacağı ancak elde edilebilecek fikri mülkiyet ve patent haklarının bu durumdan istisna olduğu kabul etmektedir. GÖNDERİCİ ve ALICI söz konusu haklarını araştırma başlangıcında karşılıklı olarak belirleyecektir.
8. ALICI bu anlaşmanın sonlanması veya biyolojik materyalin sağlandığı gönüllülere ait anlaşmanın 2. maddesinde belirtilen olurun geri çekilmesi halinde bütün materyalleri geri vermeyi veya ortadan kaldırmayı ve bunu belgelemeyi kabul eder.
9. Bu anlaşma, araştırmanın sonlanması, ilgili mevzuat hükümlerine uyulmaması veya ilgili tarafların anlaşma hükümlerine uymaması durumlarında son bulacaktır.
10. Bu anlaşmanın yürütülmesinde ALICI ve GÖNDERİCİ yetkilileri sorumludur. Anlaşmazlık halinde ihtilafın çözümü için her iki ülke mahkemeleri de yetkilidir.

BİYOLOJİK MATERYALİ GÖNDEREN ARAŞTIRMACI BİLGİSİ

Adı Soyadı ve Unvanı : Cumhur Kaan Yaltırık, Dr.Öğr.Üyesi
Uzmanlık Alanı : Beyin ve Sinir Cerrahisi
Kurumu : Yeditepe Üniversitesi Hastanesi
Adresi : İçerenköy Mahallesi Hastane Yolu Sokak no:102-104 Ataşehir-İstanbul
Telefon : 05333331800
Faks :
E-posta : dr_cky@yahoo.com

INFORMATION REGARDING THE INVESTIGATOR SENDING THE BIOLOGICAL MATERIAL

Name Surname and Title :
Specialization :
Institution :
Address :
Telephone :
Fax :
E-mail :

INFORMATION REGARDING THE CONSIGNEE RECEIVING THE BIOLOGICAL MATERIAL

Name Surname and Title :
Specialization :
Institution :
Address :
Telephone :
Fax :
E-mail :

I read and understood the terms under this agreement. I hereby agree and undertake that I will act in accordance with the terms of this agreement with respect to the dispatched materials.

	CONSIGNOR				CONSIGNEE
	Consignor Investigator	Consignor Sponsor Company Official or Legal Representative	Chief / Head of the Department	Chief Officer of the Institution / Rector or Assigned Person	Consignee Institution Official
Name Surname and Title in Handwriting					
Date					
Signature					

Note: Instead of the signature of the consignee representative, a signed "end use certificate" including clauses similar to this agreement's to be issued by the consignee institution may also be accepted.

BİYOLOJİK MATERYALİ ALAN ALICI BİLGİSİ

Adı Soyadı ve Unvanı	: Turgay İsbir, Profesör Doktor
Uzmanlık Alanı	: Biyokimya, Moleküler Tıp
Kurumu	: Yeditepe Üniversitesi
Adresi	: İnönü mah. Kayışdağı Caddesi 26 Ağustos Yerleşkesi Tıbbı Biyoloji ABD Ataşehir / İstanbul
Telefon	: 05332823726
Faks	:
E-posta	: turgay.isbir@yeditepe.edu.tr

Bu anlaşmada belirtilen koşulları okudum ve anladım. Gönderilen materyalde bu anlaşmada belirtilen koşullara uyacağımı taahhüt ederim.

	GÖNDERİCİ				ALICI
	Gönderen Araştırmacı	Gönderen Destekleyici Firma Yetkilisi veya Yasal Temsilcisi	Eğitim Görevlisi / Ana Bilim Dalı Başkanı	Kurum Amiri / Rektör veya Yetkilendirdiği Makam	Alıcı Kurum Yetkilisi
El Yazısı ile Adı Soyadı Unvanı	KAAN YAKTIRAK				Prof. Dr. Turgay İsbir
Tarih	23/08/2018				24.08.2018
İmza					

Not: Bu anlaşmada yer alan alıcı kurum yetkilisinin imzası yerine alıcı kurum tarafından verilecek olan ve içerik olarak bu anlaşmadaki hükümlere benzer hükümleri içeren imzalı "end use certificate" "son kullanım sertifikası" de kabul edilebilir.

Asgari Bilgilendirilmiş Gönüllü Olur Formu

Sayın Hastamız,

- Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.
- Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.
- Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz.** Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.
- klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da k**arar verebilmek için uygun zaman talep edebilirsiniz.**
- Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir** ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz**.
- Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşürü**nden edinebilirsiniz. Ayrıca Hastane personeli hakkında http://www.yeditepe.edu.tr web sayfasından daha detaylı bilgilere ulaşabilirsiniz.
- Burada belirtilenlerden başka sorularınız varsa bunları yanıtlamak görevimizdir.

TANIMLAMA

1. ***Araştırmanın Adı:*** **Glioblastoma Multiforme Tümörlerinde miRNA 582-5p ve miRNA 363'ün Ekspresyon Düzeylerinin Kaspaz-9 Üzerine Etkisi**

2. ***Araştırmaya Katılımcı Sayısı: 100***

Bu araştırmanın Amacı:

Primer malign beyin tümörleri 2 yıllık ortalama sağkalım süresi ile ölüme sebebiyet veren neoplaziler grubunda önemli yere sahiptir. Glioblastoma multiforme (GBM), santral sinir sisteminin en sık görülen ve en malign glial tümörüdür. GBM, malign astrositomların %80'ini oluşturur ve sağkalım süresi en kısa olan tümörlerden biridir. Standart tedavi ile tanı konulmasını takiben ortalama sağkalım süresi 14-16 aydır. Yapılan çalışmalar göstermiştir ki 3 yıldan fazla yaşam süresine sahip hastaların oranı tüm hastaların %2-3 kadarıdır.

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Son zamanlarda, miRNA'ların tümör büyümesi ve sağ kalımında kilit faktör olarak ortaya çıkması, normal beyin dokusuna kıyasla farklı tiplerdeki glioblastomlarda miRNA ekspresyon seviyelerinin değerlendirilmesine yol açmıştır. Sinir kök hücreleri (NSC) kullanılarak yapılan çalışmalar, nöral gelişimde yer alan miRNA'ların GBM gelişiminde rol oynadığını ve bilinen miRNA'ların % 70'inin beyinde ifade edildiğini göstermiştir.

Bu tez çalışmasının da hedef miRNA'ları olan miRNA 582-5p ve miRNA 363'ün onkojenik potansiyele sahip olduğu, GBM nörosfer kök hücre hatları üzerinde yapılan çalışmalarla gösterilmiştir. Ayrıca miRNA ile tedavi edilen hücre hatlarında apoptotik aktivitede bir azalma gözlemlenmiştir. Elde edilen bu sonuçlar, miRNA 582-5p ve miRNA 363'ün anti apoptotik etki mekanizmasını desteklemektedir.

Gerçekleştirilen biyoinformatik çalışmalar ve literatürden elde edilen bilgiler ışığında bu tez çalışması kapsamında; GBM tanısı almış hastalardan alınacak örnekler üzerinde, apoptotik etki mekanizması gösterilmiş olan miRNA 582-5p ve miRNA 363'ün ekspresyon seviyeleri belirlenerek, kontrol grubu ile ekspresyon düzeyi açısından bir fark olup olmadığı saptanarak araştırmaya konu olan miRNA'ların ekspresyon düzeylerinin kaspaz-9 aktivitesi üzerine olan etkisinin araştırılması hedeflenmiştir.

Süresi: ***1 Yıl***

İzlenecek Yöntem/Yöntemler:

Tez çalışması kapsamında hasta (n=50) ve kontrol (n=50) grubu olmak üzere iki gruptan oluşması planlanmıştır. Glioblastoma multiforme tanısı almış gönüllü kişilerden ve kontrol grubunu oluşturacak sağlıklı gönüllülerden kan örnekleri alınacaktır. Alınan kan örnekleri çalışmaya kadar +4°C'de saklanacaktır. iPrep DNA ekstraksiyon robotu ile kan örneklerinden genomik DNA izolasyonu yapılacaktır.

miRNA izolasyonu için hasta ve kontrol grubundan alınan kanlar 4500 rpm'de 15 dakika boyunca santrifüj edilecek ve elde edilen serumlar çalışma yapılana kadar -80'de saklanacaktır. miRNA izolasyonu için Qiagen serum plazma kiti kullanılacaktır. Elde edilen miRNA'ların saflığı için ise Nanodrop 2000 ve düzeylerinin belirlenmesi için flurometre (Qubit 3) kullanılacaktır. miRNA ekspresyon seviyesi 7500 gerçek zamanlı PZR kullanılarak ölçülecektir. Çalışmanın istatistiki analizi için SPSS 24.0 sürümü kullanılacak. Anlamlılık seviyesi $p < 0.05$ kabul edilecektir.

Araştırma Sonunda Beklenen Fayda

Glioblastoma Multiforme Tümörlerinde miRNA 582-5p ve miRNA 363'ün Ekspresyon Düzeylerinin Kaspaz-9 Üzerine Etkisinin Belirlenmesi

Bu Çalışmada Herhangi Bir Alternatif Tedavi ya da Girişimde Bulunulmayacaktır.

Bu Araştırma Gönüllüler İçin Hiçbir Risk Teşkil Etmemekte ve Hiçbir Rahatsızlığa Sebep olmamaktadır.

ONAM (RIZA)

Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğimi biliyorum. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim. Tarafımdan alınan kan ve doku örneklerinin daha sonra başka araştırma çalışmalarında kullanılmasında herhangi bir sakınca yoktur.

Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün:

Adı Soyadı:

İmzası:

Tarih:

Açıklamaları Yapan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin:

Adı Soyadı:

İmzası:

Tarih:

Gerekiyorsa Yasal Temsilcinin :

Adı Soyadı:

İmzası:

Tarih:

Klinik Araştırma Proje Koordinatörü İletişim Bilgileri:

Adı Soyadı:

Uzmanlık alanı:

Kurumu:

E-posta adresi:

Telefon numarası:

ETHICAL APPROVAL

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1561
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

25/10/2018

İlgili Makama (Deryanaz Billur)

Yeditepe Üniversitesi Biyokimya, Moleküler Tıp Anabilim Dalı Prof. Dr. Turgay İsbir'in koordinatör, Yeditepe Üniversitesi Hastanesi Beyin ve Sinir Cerrahisi Anabilim Dalı Dr. Öğr. Üyesi Cumhur Kaan Yaltırık'ın sorumlu olduğu **"Glioblastoma Multiforme Tümörlerinde miRNA 582-5p ve miRNA 363'ün Ekspresyon Düzeylerinin Kaspaz-9 Üzerine Etkisi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (**1533** kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **24.10.2018** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 916**).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı

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CURRICULUM VITAE

Personal Informations

Name	Deryanaz	**Surname**	Billur
Place of Birth	Istanbul	**Date of Birth**	12.03.1994
Nationality	T.C	**ID**	49648242830
Email	deryanazbillur@gmail.com	**Phone**	5462640330

Educational Informations

	Name of Institution	**Year**
Doctorate		
Master		
University	T.C. Istanbul Kultur University	2016
High School	FMV. Erenkoy Isık Science High School	2012

Work Experience

Responsibilty	**Institution**	**Year**
1. Scholar Master Student	Yeditepe University Medical Faculty	2018-2019
2.		
3.		

Language	**Reading***	**Speaking***	**Writing***	**KPDS/UDS Score**	**(Other) Score**
English	Very Good	Very Good	Very Good		YOKDIL 78,75
German	Basic	Basic	Basic		

*****Very Good, Good, Basic

	Quantitative		
ALES Score	70,69611		
(Other) Score			

Computer Skills

Program	**Ability to use**
Microsoft Office	Very Good

SCI, SSCI, AHCI Index Publications

Bakirezer SD, Yaltirik CK, Kaya AH, Yilmaz SG, Ozdogan S, Billur D, Isbir T. The Evaluation of Glutathione Reductase and Malondialdehyde Levels in Patients With Lumbar Disc Degeneration Disease. In Vivo. 2019 May-Jun; 33(3):811-814. doi: 10.21873/invivo.11543.