The expression of miR-513c and miR-3163 was downregulated in tumor tissues compared with margin tissues of patients with breast cancer

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Abstract

Background: Breast cancer (BC) is the most common invasive cancer with different subtypes that its metabolism is unique compared with normal cells. Glutamine is considered a critical nutrition for tumor cell growth and therefore, targeting glutamine metabolism, especially Glutaminase, which catalyzed the conversion of glutamine to glutamate can be beneficial to design anti-cancer agents. Recently, evidence has shown that miRNAs with short length and single strand properties play a significant role in regulating the genes related to glutamine metabolism and may control the development of cancer.

Methods: Since, in-silico analysis confirmed that miR-513c and miR-3163 might be involved in glutamine metabolism, the expression level of these two miRNAs was evaluated in eighty BC tissues and margin tissues. The data were analyzed to evaluate the correlation between expression level of these miRNAs and patient's characteristics such as abortion history, family history, and age. Furthermore, in-silico analysis was applied to predict the potential biological processes and molecular pathways of miR-513c and miR-3163 based on its gene targets.

Results: In-silico studies revealed the top categories of biological processes and pathways that play a critical role in cancer development were target genes for miR-513c and miR-3163. The current study showed that miR-513c (P-value= 0.02062 and fold change= -2.3801) and miR-3163 (P-value= 0.02034 and fold change= -2.3792) were downregulated in tumor tissues compared to margin tissues. Furthermore, the subgroup studies did not show any substantial relationship between expression levels of these two miRNAs and factors such as age, family history cancer, and abortion.

Conclusion: Based on our data, miR-513c and miR-3163 may be offered as a potential diagnosis and therapeutic targets for patients with BC.

1. Introduction

Breast cancer (BC) is a major health problem among female worldwide[1]. Based on the WHO statistics, this cancer impacts 2.1 million female every year [2], and causes the greatest number of cancer-related mortality among women [3]. In 2018, it is estimated that 98,755 women died from BC that is nearly 15% of all cancer mortality among females worldwide [4]. Moreover, in Iran the mean age of the women identified with this disease reduced to lower ages [5]. Breast cancer has been examined from various aspects, but the exact mechanism of it is still unclear [6]. Various subtypes of BC and their heterogeneity and complexity make some problems in the early detection and treatment of BC [7]. Many studies have suggested alteration in glutamine metabolism profile as one of the unique characteristics of BC cells; thus targeting glutamine metabolism may be important to design anti-cancer strategies against BC development [8-10].

Metabolic reprogramming is a unique event in cancer cell development [11, 12], which is utilized to supply the bioenergetic and biosynthetic demands for accelerating growth and proliferation of cancer cells [13]. Meanwhile, many cancer cells, especially BC cells are dependent on glutamine, as the major source of energy and building block, for growth and proliferation, [14, 15], and so, blocking glutamine pathway can severely influence and suppress cell proliferation [8]. Recently, valuable studies and bioinformatics analysis demonstrated that some emerged microRNAs (miRNAs) can efficiently control glutamine metabolism by targeting critical enzymes such as GLS, which can provide an opportunity for regulating cancer development [16].

MiRNAs are a class of small non-coding RNAs with 18-25 nucleotides in length, which post-transcriptionally are involved in the regulation of gene expression by degrading targeted mRNAs and/or inhibiting their translation [17, 18]. According to bioinformatics and a few experimental analyses, miR-513c and miR-3163 can regulate glutamine metabolism via targeting GLS. Regarding further experimental studies, miR-513c is a tumor suppressor in multiple cancers such as hepatocellular carcinoma [19], prostate cancer [20], glioma [21], neuroblastoma [22], breast cancer[23], and esophageal adenocarcinoma[24], whereas miR-3163 plays vital roles in retinoblastoma cancer stem cell (RCSCs) [25] and non-small

cell lung cancer cell (NSCLC) cancers [26]. Therefore, the expression level of these miRNAs can be examined to invention potential marker and their exploitation for control of glutamine metabolism[27]. Since metabolism reprogramming is a common event in cancer cells and this process significantly changes compared with normal cells [28-30], in the current study, the expression levels of both miR-513c and miR-3163 were evaluated in tumoral and margin tissues of patients with BC.

2. Methods

2.1 Selection of miRNAs

To select the most prominent enzyme in glutamine metabolism of tumor cells, the signaling pathways related to glutamine metabolism were examined. Furthermore, the articles reporting the genes involved in glutamine metabolism were reviewed. Consequently, miRNAs that target GLS were searched using miRTarBase 7.0 [19] and Target Scan Human 7.2 databases. Further, recent experimental studies were reviewed for confirmation of these miRNAs. Among numerous miRNAs, miR-513c and miR-3163 were selected because these two miRNAs were confirmed in recent studies and have revealed dysregulation in multiple cancers, especially mir-513c in BC cell lines. In this regard, we studied the expression level of miR-513c and miR-3163 in tumoral and margin tissues of patients with BC to determine whether these two miRNAs are dysregulated in this condition.

2.2 Clinical specimens

Firstly, 80 samples of tumor and margin tissues were obtained from BC patients undergoing surgery at the Noor Nejat Hospital of Tabriz. Specimens used in this study were provided from mastectomy and lumpectomy in which a portion of the tumor margin tissue was removed at a distance from the tumor tissue. In this way, the spread and invasion of cancer cells are investigated by pathological examination. In addition, tumor margin samples that were considered healthy by a pathologist are examined as the controls in the current study. Written informed consent was obtained from all the participants after enough explanation of the study, and the study was confirmed by the Ethics Committee (Ethics code: IR.TBZMED.REC.1398.025). Patients did not receive chemotherapy or radiotherapy before surgery. The specimens were collected after surgical resection, immediately frozen, and stored at -80 °C. Clinical pathological features have been shown in (Table1).

2.3 Total RNA Extraction

For extraction of total RNA, the tissue samples were homogenized with liquid nitrogen and their RNA was extracted through Trizol reagent (Geneall). Then, the quality and quantity of extracted RNAs were assessed by using NanoDrop spectrometer (Thermo Scientific, USA). After extraction, the obtained RNAs were eluted in 50 µL of RNase-free water and stored at -80 °C.

2.4 cDNA synthesis and Real-time PCR

In this study, cDNA of the miR-3163, miR-513c and RNU6 were synthesized by using reverse transcriptase enzyme (Thermo Fisher, USA), dNTP (Sinaclon, Iran), and their unique stem-loop-primers. For this purpose, three specific stem-loop primers were designed for miR-3163, miR-513c, and also RNU6 (for normalization) were employed and the conditions of PCR machines were 30 min at 16 °C, 30 min at 42 °C and 5 min at 75 °C (Table 2). For conducting Real-time PCR reaction, the SYBR Green master mix (Amplicon, Denmark), miR-3163, miR-513c specific primers and dNTP were used (Table 2). These reactions were performed by MIC PCR bimolecular system in two steps as follows: for miR-513c: 10 min at 94 °C, 40 cycles of 15 sec at 94 °C and 30 sec at 58°C. For miR-3163: 10 min at 94 °C, 40 cycles of 15 sec at 94 °C and 20s at 57 °C. Then, for RNU6 amplification :10 min at 94 °C, 40 cycles in 15sec at 94 °C and 20s at 56 °C. The results were shown by mic-PCR v1.4.0 software. The sequences of the primers are shown in table 2.

2.5 In-silico analysis

In-silico analysis was performed to evaluate biological processes and cellular pathways that are enriched by targeted genes of miR-513c-5p and miR-3163. The experimentally confirmed genes targets of miR-513c-5p and miR-3163 were obtained from the miRTarBase 6.0 database (<http://mirtarbase.mbc.nctu.edu.tw/php/index.php>) and exposed to overrepresentation enrichment analysis (ORA) based on the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes pathways using the Web Gestalt (<http://www.webgestalt.org/option.php>) webserver. In both analyses, the reference gene list and the multiple test adjustment method were set to "genome_protein_coding" and Benjamini-Hochberg, respectively. In the GO analysis, the 10 top important categories were selected. Other parameters were set as defaults.

2.6 Statistical analysis

All of the experiments were performed at least two times. Shapiro-Wilk test was checked whether data are normally distributed or not. Paired t-test and delta-CTs are used for comparing the gene expressions between tumor tissues and margins. Two sample t-test and logarithm2 of fold applied to compare expression alterations of genes in tumors relative to margins tissue across subsets of study samples. All statistical analyses were conducted using R software version 0.5. 1. Finally, $p < 0.05$ was considered statistically significant.

3. Results

Based on miRTarBase, miRDB, and Target Scan Human 7.2, as miR- related databases, miR-513c and miR-3163 were selected as potential miRNAs that may play a significant role in the regulation of glutamine metabolism pathway via targeting GLS. Based on recent experimental studies, miR-513c has tumor-suppressive roles, while miR-3163 plays diverse roles in several cancers [23, 25, 31]. To determine the roles of miR-513c and miR-3163 in human breast cancer, we compared the expressions of both miRNAs in BC tumor tissues and their margin tissues were obtained from patients undergoing surgery at the Noor Nejat Hospital of Tabriz.

3.1 MiR-513c and miR-3163 are downregulated in breast cancer tissues

3.1.1 MiR-513c

The results of paired T-test show that the expression level of miR-513c was relatively downregulated in human breast cancer tissues compared to margin tissues (p -value= 0.02062) and (fold change= -2.3801) (**Figure 1a**).

3.1.2 MiR-3163

The results of paired t-test reveal that expression level of miR-3163 was relatively downregulated in human breast cancer tissues compared to margin tissues (p -value=0.02034) and (fold change=-2.3792) (**Figure 1b**).

3.2 Subgroups analysis

To show the relationship between the expression of miR-513c and miR-3163 with factors such as age, family history cancer, and abortion in breast cancer subgroups, sample t-test of logarithm2 of fold changes were employed.

3.2.1 MiR-513c

We did not find any significant changes in the expression of miR-513c in age subgroups of ≤ 50 years and > 50 years (p -value =0.6758) and (fold change=-0.42103) (**Figure 2 left**). Additionally, our analysis has been proved, there is no

significant relationship between the expression of miR-513c with abortion (p-value= 0.7713) and (fold change =0.29275) (**Figure.2 middle**) and cancer family history subgroups (p-value =0.0525) and (fold change =-1.9859) (**Figure.2 right**).

3.2.2 MiR-3163

Based on results of paired sample t-test, there is no markedly changes in the expression level of miR-3163in subgroups cancer family history (p-value = 0.1208), (fold change = -1.5756) (**Figure.3 left**). Additionally, our data showed that there is no significant relationship between the expression of miR-3163 with abortion history (p-value = 0.5634), (fold change = 0.58281) (**Figure 3 middle**), and age in patients ≤ 50 and < 50 (p-value = 0.3805), (fold change = 0.8859) (**Figure 3 right**).

3.3 In-silico analysis

A total of 98 and 333 experimentally proved target genes for miR-513c and miR-3163 were extracted from the miRTarBase database, respectively. The miRNA-gene interaction was portrayed by Cytoscape V 3.7.1 (**Figure 3, 4**). The ORA showed that significant categories of biological procedures were overrepresented among target gene of miR-513c and miR-3163 (**Table 3, 4**). Pathway analysis revealed top biological enriched-pathways and processes in target genes of miR-513c and miR-3163 (**Table 5, 6**). Based on these analysis, target genes of miR-513c are involved in pathways related to cancer development. Furthermore, including substantial biological processes were enriched among target genes of miR-3163, such as negative regulation of nucleobase-containing compound metabolic process, regulation of mRNA metabolic process, positive regulation of nucleobase-containing compound metabolic process, negative regulation of RNA metabolic process, and negative regulation of cellular macromolecule biosynthetic process are associated to metabolism pathways. Also, among several cellular pathways that are considerably influenced by miR-3163; MAPK, Hedgehog, Wnt signaling pathways are the most significant pathways. These biological processes and cellular pathways that are related to BC progression and cancer metabolism.

4. Discussion

MicroRNAs have been proved as hopeful biomarkers in the diagnosis and prognosis of BC [32]. Irregular expression of miRNAs impacts the processes involved in the development of BC such as invasion, metastasis, promoting tissue, stimulating antiapoptotic activity, drug resistance, and metabolism reprogramming [18, 33, 34]. These critical molecules have been found as key players in cancer metabolism by regulating genes related to metabolism pathways[35]. According to bioinformatics databases, miRNAs such as mir-513c and miR-3163 are involved in glutamine metabolism, which have been examined in the present work. Our outcomes demonstrate that mir-513c and mir-3163 have been downregulated considerably in BC tissues compared to margin tissues.

To the best of our knowledge, the downregulation and tumor suppressor role of miR-513c in multiple cancers has been proved. In R2N1d and MDA-MB-231 breast cancer cell lines treatment with HDACi , among the most considerably expressed miRNAs, mir-513c also emerged as the most regularly upregulated and act as a tumor suppressor, in induction cell death process [23]. Also, in hepatocellular carcinoma and glioblastoma (GBM), mir-513c is strictly downregulated and overexpression this miRNA prevented the proliferation of these cancer cells through targeting MET and Wnt/ β -catenin signaling pathway, respectively [19] [21]. Additionally, in neuroblastoma, mir-513c is markedly downregulated. Moreover, mir-513c as a tumor suppressor plays an important role in regulating glutamine metabolism by targeting GLS [22].

Since the role of miR-513c-5p has not been fully understood, bioinformatics analysis was done to shed the light on molecular pathways and biological procedures that are potentially impacted by dysregulation of miR-513c-5p. In-silico studies proved that target genes of miR-513c are involved in pathways related to cancer progression. In the present study, miR-513c was relatively downregulated in BC tissues compared with margin tissues (p-value= 0.02062, Fold change=-2.3801)

. Based on our research, several studies have been carried out on mir-513c, but this is the first study that was done on clinical samples of BC. Therefore, it seems mir-513c may act as a tumor suppressor and offers as a considerable marker in diagnostic and prognostic aims of BC patients.

In several studies, the activity and expression of mir-3163 in multiple cancers such as NSCLC and Retinoblastoma (RB) have been evaluated. In NSCLC, mir-3163 as a moderator contributes to Meg3 to suppress and regulate the translation of *skp2*. However, mir-3163 expression does not differ in NSCLC compared with normal cells, which suppresses the *skp2* translation and reduction of its level in corporation with Meg3 to decrease cell proliferation in NSCLC [26]. Also, in Retinoblastoma (RB), upregulation of mir-3163 via targeting ABCG2, reduced the multidrug resistance and promotion of apoptosis in RCSC [25].

Because the activity of miR-3163 has not been completely understood, bioinformatics analyses were performed to determine a significant relationship between cancer and miR-3163. This analysis provides an insight into molecular pathways and biological processes that are potentially regulated by target genes of miR-3163. Based on this analysis, among important biological processes, negative regulation of cellular macromolecule biosynthetic process (GO: 2000113) is associated to metabolism pathways[36]. Also, including significant cellular pathways enriched by miR-3163, which are related to cancer cells progression, such as MAPK signaling pathway, Hedgehog signaling pathway, and Wnt signaling pathway are the most important. MAPK and Hedgehog signaling pathways are critical key regulators in cellular functions such as cell differentiation, proliferation, differentiation, survival, and apoptosis. The higher activation of MAPK in subtypes of BC predicts invasive phenotypes and poor prognosis [37-39]. Furthermore, these signaling pathways involve in the regulation of glutamine metabolism pathway [40] [41, 42]. Also, Wnt signaling pathway as a master regulator, plays a significant role in the progression and development of BC by metabolism reprogramming [43] [44]. So, this information may provide a valuable clue about cancer metabolism and our preliminary theory.

In the present study, our data contrary to previous studies have proved that the expression of mi-3163 was relatively downregulated in BC tissues compared with margin tissues (p-value=0.02034, Fold change= -2.3792). Recent reports have shown that miR-3163 plays diverse roles in various cancers; nonetheless, further studies are needed to identify and validate the precise role of this microRNA. Based on our results and bioinformatics analyses, it seems that miR-3163 can play the master role in development of BC biology.

Overall, based on our analysis, no significant association was found between miR-513c and miR-3163 expression and variables such as age, cancer family history, and abortion.

5. Conclusion

Altogether, based on our results, mir-513c and mir-3163 expression are relatively downregulated in breast cancer tissues in comparison to margin tissues (p-value= 0.02062, Fold change= -2.3801) (p-value=0.02034, Fold change= -2.3792) respectively. Therefore, it seems that mir-513c and mir-3163 probably serve as tumor suppressors in patients with BC. Additionally, according to our data, there is not a remarkable association between the expression of both miRNAs with factors such as age, abortion history, and cancer family history. However, further studies are needed to consider these miRNAs as therapeutic targets and elucidate molecular mechanisms and validate bioinformatics and predicted results.

Abbreviations

BC, Breast Cancer; GLS, Glutaminase; HCC, hepatocellular carcinoma; LPR6, Low-density lipoprotein (LDL) receptor-related protein-6; RB, Retinoblastoma; GBM, Glioblastoma; ABCG2, ATP Binding Cassette Subfamily G Member 2; MEG3, Maternally Expressed 3; RCSCs, Retinoblastoma Cancer Stem Cells; NSCLC, Non-small cell lung cancer. HDACi; Histone deacetylase inhibitor, WHO, World Health Organization;

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran with Ethics code IR.TBZMED.REC.025.

Consent for publication: Not Applicable

Availability of data and materials: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Competing Interests: The authors declare no conflict of interest.

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Author's contribution: Soheila Delgir designed the study. Mohammad Reza Alivand and Farhad Seif wrote the first draft of the manuscript. Milad Bastami, Asma Safei, and Khandan Ilkhani gathered the data. Milad Bastami and Mohammad Reza Alivand supervised the study. All authors read and approved the final version of the manuscript.

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Tables

Table 1
Basis characteristics of the patients with breast cancer.

Parameter	Number (percentage)
Tumor grade	Grade 3 = 5%
	Grade 2 = 87.5%
	Grade 1 = 7.5%
Lymph node	Yes = 87.5%
	No = 12.5%
Family Cancer History	Yes = 47.5%
	No = 41.3%
	Unknown = 11.2%
Abortion History	Yes = 36.2%
	No = 51.3%
	Unknown = 12.5%
Age	=<50 38.8%
	> 50 40%
	Unknown = 21.2%

Table 2
cDNA synthesis specific stem-loop primers and Real-time PCR primer sequences.

	Noncoding-RNAs and their accession numbers	Sequences	
cDNA synthesis reaction	MiR-3163-5p NIMAT 0015037	MiR-3163(STL)	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACGTCTTAC-3'
	Mir-513c-5p NIMAT 0022728	Mir-513c(STL)	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACATAAAC-3'
	RNU6 NR_003027.2	U6 (STL)	5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACAAAAATAT-3'
Real time PCR reaction	MiR-3163-5p NIMAT 0015037	Mir-3163(F)	5'-AGGGTATAAAATGAGGGCAGTAAGAC-3'
	Mir-513c-5p NIMAT 0022728	Mir-513c(F)	5'-GGGTTCTCAAGGAGGTGTCG-3'
	MiR-3163& Mir-513c	Common (R)	5'-GTGCAGGGTCCGAGGT-3'
	RNU6	U6 (F)	5'-GCTTCGGCAGCACATATACTAAAAT-3'
	NR_003027.2	U6 (R)	5'-CGCTTCACGAATTTGCGTGTCAT-3'

Table 3
Pathways that are enriched among target genes of mir-513c.

No	ID	Pathway name	O	FDR	E	P-value
1	hsa05222	Small cell lung cancer	92	1	0.34163	0.0046026
2	hsa00130	Ubiquinone and other terpenoid-quinone biosynthesis	11	1	0.040847	0.040124
3	hsa05200	Pathways in cancer	524	1	1.9458	0.041062
4	hsa05032	Morphine addiction	91	1	0.33792	0.044411
Abbreviations: E, number of expected genes in each category; O, number of observed target genes in each specific category.						
aThe Benjamini-Hochberg adjusted P-value.						

Table 4
Pathways that are enriched among target genes of miRNA-3163

Nos	Gene set	Pathway name	C	E	R	FDR	P-value
1	hsa05170	Human immunodeficiency virus 1 infection	212	3.6155	3.3191	0.081094	2.5E-04
2	hsa04110	Cell cycle	124	2.1147	3.783	0.09676	1E-03
3	hsa04114	Oocyte meiosis	124	2.1147	3.783	0.09676	1E-03
4	hsa05202	Transcriptional misregulation in cancer	186	3.1721	3.1525	0.09676	1E-03
5	hsa04010	MAPK signaling pathway	295	5.0309	2.584	0.09676	1.5E-03
6	hsa05167	Kaposi sarcoma-associated herpesvirus infection	186	3.1721	2.8373	0.2378	4.4E-03
7	hsa04340	Hedgehog signaling pathway	47	0.80154	4.9904	0.3802	8.2E-03
8	hsa04310	Wnt signaling pathway	146	2.4899	2.8114	0.49589	1E-02
9	hsa05206	MicroRNAs in cancer	150	2.5581	2.7364	0.50585	1.4E-02
10	hsa04218	Cellular senescence	160	2.7286	2.5654	0.58131	2E-02
Abbreviations: C, the total number of genes in the category; E, number of expected genes in each category; R, fold enrichment.							
aThe Benjamini-Hochberg adjusted P-value							

Table 5
Top ten enriched biological processes among target genes of miR-513c.

No	GO ID	Biological process	O	FDR	E	R	P-value
1	GO:0042148	strand invasion	5	1	0.024004	83.32	2.2E-04
2	GO:0006612	protein targeting to membrane	187	1	0.89774	6.6834	2.7E-04
3	GO:0045047	protein targeting to ER	107	1	0.51368	7.7869	1.7E-03
4	GO:0072599	establishment of protein localization to endoplasmic reticulum	111	1	0.53289	7.5063	1.9E-03
5	GO:0008340	determination of adult lifespan	17	1	0.081613	24.506	2.9E-3
6	GO:0006605	protein targeting	412	1	1.9779	3.5391	3.6E-03
7	GO:0090150	establishment of protein localization to membrane	313	1	1.5026	3.993	3.9E-03
8	GO:0070972	protein localization to endoplasmic reticulum	137	1	0.65771	6.0818	4.2E-03
9	GO:0031297	replication fork processing	95	1	0.15362	13.019	1E-02
10	GO:0006614	SRP-dependent co-translational protein targeting to membrane	95	1	0.45607	6.5779	1E-02
Abbreviations: C, the total number of genes in the category; E, number of expected genes in the category; GO, Gene Ontology; R, fold enrichment; O, number of observed target genes in each specific category.							
aThe Benjamini-Hochberg adjusted P-value							

Table 6
Top ten enriched biological processes among target genes of miR-3163

No	Gene set	Biological pathways	C	E	R	FDR	P-value
1	GO:0045934	negative regulation of nucleobase-containing compound metabolic process	1418	22.805	1.9732	0.04352	8E-06
2	GO:0033554	cellular response to stress	1867	30.026	1.7984	0.04352	1E-05
3	GO:0010629	negative regulation of gene expression	1733	27.871	1.8299	0.04352	1E-05
4	GO:1903311	regulation of mRNA metabolic process	266	4.278	3.5063	0.06326	3E-05
5	GO:0045935	positive regulation of nucleobase-containing compound metabolic process	1847	29.705	1.7169	0.12664	8E-05
6	GO:0000278	mitotic cell cycle	927	14.909	2.0793	0.12664	9E-05
7	GO:0051253	negative regulation of RNA metabolic process	1295	20.827	1.8726	0.12664	1E-04
8	GO:0000902	cell morphogenesis	982	15.793	2.0262	0.12664	1E-04
9	GO:0032989	cellular component morphogenesis	1082	17.401	1.9539	0.12664	1E-04
10	GO:2000113	negative regulation of cellular macromolecule biosynthetic process	1359	21.856	1.8301	0.12664	1E-04

Figures

Figure 1a

Figure 1b

Figure 1

left. Boxplot of cancer expression level of mir-513c and age, patients >50 (n=31), patients ≤50 (n=31), age unknown for (n=18) cases. (P-value=0.6758). Middle. Boxplot of abortion history and expression level of miR-513c. Patients without abortion history (n=41), patients with abortion history (n=29), unknown abortion history (n=10). (P-value=0.7713) right. Boxplot of cancer expression level of mir-513c and family history. Patients with family history (n=38), patients without family history (n=33), unknown family history (n=9) (P-value=0.0525).

Figure 2

Left. Boxplot of cancer family history and expression level of mir-3163. Patients with family history (n=38), patients without family history (n=33), unknown family history (n=9). (p-value= 0.1208). Middle. Boxplot of abortion history and expression level of miR-3163. Patient without abortion history (n=41), patients with abortion history (n=29), unknown abortion history (n=10). (p-value= 0.5634). Right. Boxplot of ages and expression level of miR-3163. Patients >50 (n=31), patients <=50 (n=31), age unknown for (n=18) cases. (p-value= 0.3805).

Figure 3

The interaction between miR-513c-5p and its target genes.

Figure 4

The interaction between miR-3163 and its target genes.