

mRNA-miRNA integrative analysis of diabetes-induced cardiomyopathy in rats

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1. ABSTRACT

An integrative analysis of miRNA and mRNA expression profiles in left ventricle (LV) of diabetes-induced rats was performed to elucidate the role of miRNAs and their mRNAs target in diabetic cardiomyopathy (DCM). mRNA (GSE4745) and miRNA (GSE44179) datasets were downloaded from Gene Expression Omnibus 2R (GEO2R) and differentially expressed mRNAs and miRNAs were selected. Cardiotoxicity-related mRNAs (n=7) were analyzed by Ingenuity Pathway Analyses 6 (IPA) and regulatory miRNAs (n=639) were identified using TargetScan 7.1. web dataset. The integrative analysis was performed between miRNAs differentially expressed in GSE44179 and regulatory TargetScan-detected miRNAs of mRNAs differentially expressed in GSE4745. *Pla2g2a* and *Hk2* mRNAs were up-and-down regulated, respectively, in GSE4745 on days 3 and 42 after diabetes-induction. The *Pla2g2a* regulatory miRNAs, rno-miR-877, rno-miR-320 and rno-miR-214, were down-regulated, and *Hk2* regulatory miRNAs,

rno-miR-17, rno-miR-187, rno-miR-34a, rno-miR-322, rno-miR-188, rno-miR-532 and rno-miR-21, were up-regulated in GSE44179 dataset. These results are suggestive that *Pla2g2a* and *Hk2* mRNAs and their regulatory miRNAs play a role in DCM pathogenesis and they may be potential circulating biomarkers to detect early cardiovascular complications in diabetic patients.

2. INTRODUCTION

Diabetes is a group of heterogeneous metabolic disorders which has in common the hyperglycemia triggered by a defect in insulin action and/or secretion (1). Diabetes is one of the most common metabolic disorders in the world and its prevalence in adults has been increasing in the last decades (2,3). In 2013, 382 million people had diabetes; this number is expected to rise to 592 million by 2035 (2).

Chronic hyperglycemia leads to severe complications including microvascular and macrovascular diseases that may affect the life quality and survival rate of diabetic patients (1). The most common microvascular complications are diabetic nephropathy, peripheral neuropathy, autonomic neuropathy and retinopathy, while the macrovascular diseases include cerebrovascular disease, coronary artery disease (CAD) and peripheral vascular disease, which have an important relationship with atherosclerosis and diabetic cardiomyopathy (DCM) (1,4,5).

DCM has three important heart alterations associated with diabetes: 1) systolic and diastolic left ventricle (LV) dysfunction regardless of hypertension, CAD and other heart diseases (6); 2) evidence of myocardial structural and functional changes, such as myocardial hypertrophy, fibrosis and necrosis (7); and 3) heart failure (8). Additionally, metabolic disorders, such as depletion of glucose transporter 4, insulin resistance, increased free fatty acids, carnitine deficiency and alterations in calcium homeostasis, as well as myocardial fibrosis, small vessel disease and cardiac autonomic neuropathy and are involved in the pathogenesis of DCM (9–11).

Studies using diabetes-induced animal models have also shown structural, functional and molecular alterations in LV related to the hyperglycemic status (12–14). Insulin-like growth factor 1 receptor activation was shown to prevent diabetes-induced cardiac fibrosis and diastolic dysfunction (12). Inflammatory condition is triggered by hyperglycemia in diabetic patients, and has been associated with an over expression of many inflammatory mediators in LV, such as tumor necrosis factor- α , interleukin 1 β (13) and transforming growth factor β (TGF β) (14). Also, in the hyperglycemic condition, the levels of the collagen degrading matrix metalloproteinase 2 (MMP-2) decreases, suggesting that normalizing the MMP-2 activity is possible to prevent cardiac fibrosis in STZ-induced cardiomyopathy (14).

Molecular mechanisms involved in DCM physiopathology are not completely elucidated. It has been reported that hyperglycemia-induced BNIP3 expression, a protein involved in mitochondrial function and apoptosis in the heart, may compromise cardiac cell survival and function (15). On the other hand, up-regulation of rno-miR-1 and rno-miR-206 related to down-regulation of Hsp60 mRNA target, in myocardium rats, neonatal ventricular cardiomyocytes, and H9C2 cells exposed to high levels of glucose, were associated with cardiomyocyte apoptosis (16). These studies provided relevant information to understand some of the genes involved in DCM mechanisms. However, a systematic biology approach has not been used to investigate a broader range of genes that could be involved in DCM physiopathology.

The systematic biology approach using an *in silico* analysis (17) may be an alternative approach in order to expand the comprehension of miRNA-regulated mRNA differential expression and its role in the development of DCM. Integrative analysis evaluating miRNA-mRNA target interactions has been performed to elucidate the pathogenesis of some diseases. Through *in silico* analysis using datasets of platelet miRNA and blood mRNA expression profiles of CAD in patients and healthy controls available at Gene Expression Omnibus (GEO) platform, investigators analyzed the prediction of miRNAs to target mRNAs dysregulated in CAD such as, *TFEC*-has-miR-545 and *hsa-miR-585-SPOCK1* (18).

Thus, the interactions between miRNAs and mRNAs differentially expressed in LV of diabetes-induced rats were evaluated using bioinformatic tools, aiming to identify mRNAs and their regulatory miRNAs, which play a role in DCM pathogenesis.

3. METHODS

3.1. Study design and experimental models

mRNA and miRNA expression profiles (GSE4745 and GSE44179, respectively) were downloaded from GEO datasets (19). GSE4745 is a microarray dataset of global mRNA expression in ventricles of Wistar rats with diabetes induced by streptozotocin (STZ) at three time points: 3 days (n=4), 28 days (n=4) and 42 days after STZ injection (n=4). Control rats were injected with citrate buffer at the same time points: 3 (n=4), 28 (n=4) and 42 (n=4). Diabetic rats showed increased blood glucose 4.3., 5.6. and 5.0. fold, and ventricle weight/body weight ratio of 0.9.1, 0.9.9 and 1.3.5 fold, respectively, at 3, 28 and 42 days after STZ-induction compared to controls (p-value <0.0.5). mRNA expression was analyzed by the microarray system using the platform: GPL85 (RGU34A) Affymetrix Rat Genome U34 Array. The investigators that performed GSE4745 data published a study which investigated the conversion of acyl-carnitine in a long-chain free fatty acid and the exported system in mitochondria of STZ-diabetic rat hearts (20).

GSE44179 is a microarray dataset of global miRNA expression in LV of diabetic (n=4) and non-diabetic (n=2) Wistar rats. Diabetes was induced by STZ followed by high fat diet for 12 weeks. Control rats (n=2) were fed with regular chow and citrate buffer injection. The diabetes rats' blood levels of glucose (556 ± 108 mg/dL), total cholesterol (140 ± 55 mg/dL) and triglycerides (79 ± 21 mg/dL) were higher in diabetic than in control rats at the time of euthanasia (p-value <0.0.5). Intraperitoneal glucose tolerance test and Intraperitoneal insulin resistance test confirmed the glucose intolerance and insulin resistance in

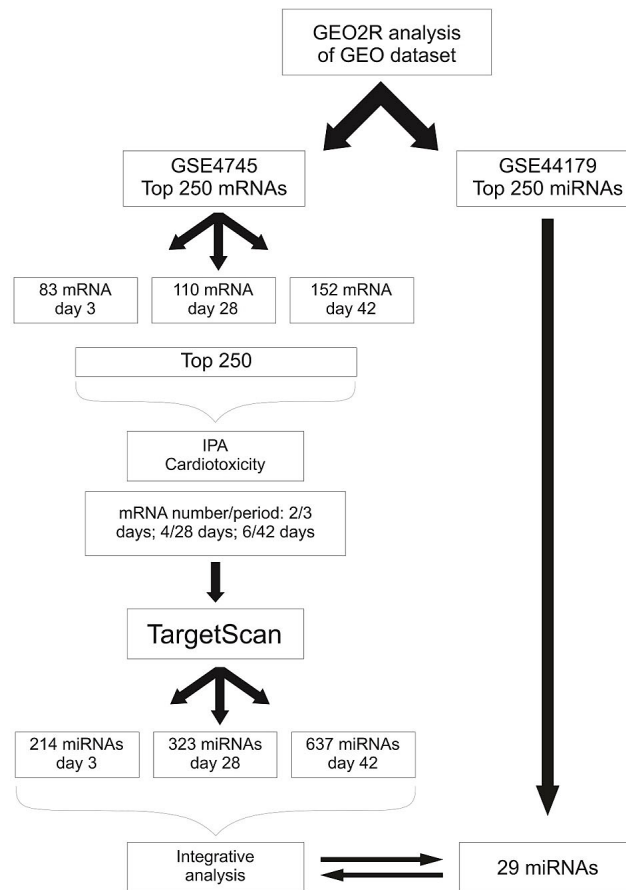


Figure 1. *In silico* analysis strategy of mRNA-miRNA differential expression data associated with diabetes-induced cardiotoxicity. Left ventricle of STZ-induced diabetic rats was used for microarray analysis. GEO2R: Gene Expression Omnibus 2R software; GEO, Gene Expression Omnibus; IPA, Ingenuity Pathway Analyses 6; MICRORNA, microRNA database.

diabetic rats. DCM in rats was demonstrated by 1.6.-fold increase in a heart-to-body weight ratio and the presence of non-ischemic lesions, interstitial and perivascular fibrosis as well as myocytes atrophy and hypertrophy. miRNA expression was analyzed by the microarray system using the platform: GPL14613 (miRNA-2_0) Affymetrix Multispecies miRNA-2_0 array. The investigators that performed GSE44179 data evaluated the role of *Cdc42* and *Pak1* genes and rno-miR-30c in rats with DCM (21). The *in silico* analysis strategy used in this study is described in Figure1.

3.2. Microarrays data processing

Rat LV mRNA expression from GSE4745 dataset was compared between diabetic and control rats of each experimental period (3, 28 and 42 days after STZ diabetes induction), using Gene Expression Omnibus 2R (GEO2R). GEO2R (22) is a tool which allows comparison of data from two or more groups of samples in order to identify genes that are differentially expressed in experimental conditions. Three top lists of mRNAs differentially and significantly expressed

($|FC| > 2$; $p\text{-value} < 0.05$) at each study period were selected. Additionally, when the mRNAs that were repeated in the top list were considered, only the mRNA with the best p-value and when p-value or FC were out of the cutoff, the mRNAs were not evaluated.

The list of top LV miRNAs differentially expressed ($|FC| > 2$; $p\text{-value} < 0.05$) of diabetic and control rats from GSE44179 dataset were also selected using GEO2R. The repeated miRNAs of the top list were considered the only one which had the best p-value and when P-value or FC was out of the cutoff, the miRNAs were not evaluated.

3.3. DCM-related gene study

The mRNAs differentially expressed were filtered by $|FC| > 2$ and $p\text{-value} < 0.05$ after the analysis by GEO2R and were uploaded into the *Ingenuity Pathway Analyses 6* (IPA) software, to identify interactions between genes and to create networks including upstream regulators, signaling pathways, physiological systems and pathophysiological

Table 1. DCM-related mRNAs differentially expressed in left ventricle of STZ-induced diabetic rats

Period	Categories	Dysfunctions	mRNAs	p-value
Day 3	Cardiac Necrosis/Cell Death	Survival Of Ventricular Myocytes	Hk2 ¹	0.03190
	Cardiac Fibrosis	Perivascular Fibrosis	Pla2g2a ¹	0.00462
Day 28	Cardiac Necrosis/Cell Death	Survival Of Ventricular Myocytes	Hk2 ¹	0.04030
		Apoptosis Of Cardiomyocyte	Nppa, Txnip	0.06020
	Cardiac Fibrosis	Perivascular Fibrosis	Pla2g2a ¹	0.00585
	Cardiac Hypertrophy	Hypertrophy Of Cardiomyocyte	Agtr1, Nppa	0.04400
Day 42	Cardiac Necrosis/Cell Death	Survival Of Ventricular Myocytes	Hk2 ¹	0.04340
		Cell Death Of Cardiomyocytes	Fstl1, Hspb1, Nppa, Txnip	0.00198
		Apoptosis Of Cardiomyocytes	Fstl1, Nppa, Txnip	0.00915
		Apoptosis Of Ventricular Myocytes	Fstl1	0.05550
	Cardiac Fibrosis	Perivascular Fibrosis	Pla2g2a ¹	0.00632
	Cardiac Hypertrophy	Hypertrophy Of Cardiomyocytes	Agtr1, Nppa	0.05050

The relationship between cardiac dysfunction and DCM-related mRNAs carried out by IPA software using mRNAs downloaded from GSE4745 dataset. ¹mRNAs differentially expressed in three time points (*Hk2* and *Pla2g2a*). DCM: diabetic cardiomyopathy

processes. The IPA-cardiac functional tool was used to select the genes involved in cardiotoxicity (hypertrophy, fibrosis and necrosis/cell death) and to generate a summary list of DCM-related mRNAs differential expression for each period of exposure to STZ, according to GSE4745 dataset.

3.4. Regulatory miRNAs of DCM-related mRNAs

TargetScan 7.1. web dataset (23) was used to search the regulatory miRNAs of the DCM-related mRNAs differentially expressed LV of rats at the three periods of the STZ treatment. The analysis uses algorithms for target site predictions based on miRNA-mRNA interactions and provides a context++ model of miRNA target efficacy which is the more predictive than any published model and at least as predictive as the most informative *in vivo* crosslink approaches. The miRNA-mRNA interactions according to IPA software and TargetScan 7.1. web dataset were constructed in the IPA tool.

3.5. Integrative mRNA-miRNA analysis

The integrative analysis was performed using the miRNAs differentially expressed in GSE44179 dataset and the miRNAs selected by the TargetScan 7.1. web dataset predictive tools, as having a regulatory role on GSE4745 differentially expressed mRNAs. The Venn diagram was used to evaluate the number of miRNAs commonly found in both sets of miRNAs.

3.6. Statistical analysis

The Limma (linear models for microarray data) was used to summarize the results of the linear model, perform hypothesis tests and to adjust the p-values

for multiple testing (24). The Fold-Change (FC) is a measuring of the changes in the expression level of a gene, and it was considered significant when $|FC| > 2.0$. between two experimental conditions (control and diabetic samples in both datasets). The *Benjamini & Hochberg false discovery rate* method was selected because it is the most commonly used method for adjustment of p-values for analysis of microarray data and provides a good balance between discovery of statistically significant genes and limitation of false positives; p-value < 0.05 was considered significant. The context++ score generated by TargetScan 7.1. web dataset is a method used to predict miRNA target efficacy (23).

4. RESULTS

4.1. mRNA and miRNA differentially expressed in rat LV

The analysis of mRNAs differentially expressed in LV of STZ-induced diabetic rats resulted in 3 lists of 85, 113 and 154 mRNAs that were differentially and significantly expressed considering $|FC| > 2$; p-value < 0.05 , significant values, respectively, at 3, 28 and 42 days after STZ induction, compared to control rats (Table 3).

The data from GSE44179 showed that 29 miRNAs were differentially and significantly expressed ($|FC| > 2$; p-value < 0.05) in LV of rats with diabetes induced by high fat diet and STZ in comparison with non-diabetic rats. Eleven miRNAs were down-regulated: rno-mir-122, rno-mir-184, rno-mir-151, rno-miR-194, rno-mir-22, rno-mir-214, rno-miR-30-c2*, rno-mir-320, rno-mir-204, rno-mir-342 and rno-mir-877; and eighteen were up-regulated: rno-mir-34a,

Table 2. DCM-related regulatory miRNAs and mRNAs targets differentially expressed in left ventricle of STZ-induced diabetic rats

GSE44179 data set			GSE4745 dataset				
Regulatory miRNAS	logFC	p-value	Target mRNAS	Period (days)	Dysfunction	LogFC	p-value
rno-miR-184	-2.09↓	0.03708	Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
rno-miR-17-3p	1.647↑	0.01334	Fstl1	42	CN	-1.328↓	0.00013
			Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
			Txnip	42	CN	1.617↑	0.00006
rno-miR-187	1.355↑	0.02161	Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
rno-miR-34a	2.606↑	0.00424	Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
rno-miR-322	1.433↑	0.00451	Fstl1	42	CN	-1.328↓	0.00013
			Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
			Txnip	42	CN	1.617↑	0.00006
rno-miR-214	-1.25↓	0.01773	Fstl1	42	CN	-1.328↓	0.00013
			Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
			Pla2g2a	3	CF	1.050↑	0.00576
				28	CF	-1.045↓	0.00071
				42	CF	1.735↑	0.00009
			Txnip	42	CN	1.617↑	0.00006
rno-miR-188	1.091↑	0.04111	Agtr1	28	CH	2.019↑	0.00064
			Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
rno-miR-532	2.149↑	0.04686	Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
			Txnip	42	CN	1.617↑	0.00006
rno-miR-21	2.133↑	0.00009	Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004

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rno-miR-320	-1.18↓	0.02567	Fstl1	42	CN	-1.328↓	0.00013
			Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
			Pla2g2a	3	CF	1.050↑	0.00576
				28	CF	-1.045↓	0.00071
				42	CF	1.735↑	0.00009
rno-miR-342	-1.08↓	0.01603	Txnip	42	CN	1.617↑	0.00006
			Fstl1	42	CN	-1.328↓	0.00013
			Hk2	3	CN	-1.882↓	0.00389
				28	CN	2.153↑	0.00001
				42	CN	-4.159↓	0.00004
rno-miR-877	-1.01↓	0.02886	Txnip	42	CN	1.617↑	0.00006
			Pla2g2a	3	CF	1.050↑	0.00576
				28	CF	-1.045↓	0.00071
				42	CF	1.735↑	0.00009

CN: cardiac necrosis and cell death; CF: cardiac fibrosis; CH: cardiac hypertrophy; DCM: diabetic cardiomyopathy; logFC: Log2-fold change. Negative and positive logFC values indicate down (↓) and up (↑) regulation, respectively.

Table 3. Differentially expressed mRNAs in left ventricle at 3, 28 and 42 day after STZ-induction

Day 3				Day 28				Day 42			
mRNA	FC	logFC	p-value	mRNA	FC	logFC	p-value	mRNA	FC	logFC	p-value
Acot1	14.0160	3.809	0.00129	Acot1	18.1136	-4.179	0.000155	Hmgcs2	134.9237	7.076	0.000000002
Cyp2e1	7.5632	2.919	0.00455	Hmgcs2	14.7128	-3.879	0.000517	Hspa1b/1a	102.1822	-6.675	0.000000057
Myh7	7.2200	2.852	0.00018	Cyp7a1	8.7362	3.127	0.001110	Acot1	92.7966	6.536	0.000001060
Spta1	7.0031	2.808	0.01359	Hmgcs2	7.4178	-2.891	0.000001	Alox15	25.8304	4.691	0.000000768
Pygl	6.3423	-2.665	0.00464	Dlg2	5.3591	-2.422	0.010300	Hspa1b/1a	24.4710	-4.613	0.000000229
Hmgcs2	5.6451	2.497	0.00003	Alox15	5.1982	-2.378	0.002760	Hmgcs2	23.9342	4.581	0.000000004
Myc	4.7272	2.241	0.00425	Tgm1	5.1946	-2.377	0.000153	Cyp2e1	23.0229	4.525	0.000038800
Penk	4.6719	2.224	0.00204	Pgf	4.9041	2.294	0.004670	Hspa1b/1a	21.9477	-4.456	0.000000274
Scg5	4.2018	-2.071	0.01159	Serpib3a	4.9007	2.293	0.001250	Hk2	17.8642	-4.159	0.000043800
Kif4a	4.1382	-2.049	0.01291	Pik3c2g	4.7833	-2.258	0.000002	Hspa1b/1a	16.6910	-4.061	0.000002060
Vps33b	4.1325	2.047	0.00891	Gpam	4.7240	-2.240	0.002250	Hspa1a	15.7579	-3.978	0.000000133
Pik3r1	3.9917	-1.997	0.00870	Gpam	4.7076	-2.235	0.000095	Dbp	14.2313	-3.831	0.000000187
Cdk1	3.9504	-1.982	0.00032	Sctr	4.6332	2.212	0.009440	H19	12.2355	3.613	0.000000289
Gdf10	3.8960	1.962	0.00864	C2cd4b	4.6044	-2.203	0.000010	Rt1ba	10.2461	-3.357	0.000000229
Ddias	3.8825	1.957	0.00412	Pc	4.5726	2.193	0.000679	Cyp26b1	9.8015	3.293	0.000002470
Pik3c2g	3.6884	1.883	0.00150	Csap1	4.5253	2.178	0.007290	Cebpd	8.3166	3.056	0.000213000
Hk2	3.6859	-1.882	0.00389	Hk2	4.4475	2.153	0.000006	Rt1da	7.9173	-2.985	0.000005780
Pdzd4	3.4750	1.797	0.00126	Cbr1	4.0954	-2.034	0.000007	Dbp	7.6529	-2.936	0.000000179
Muc5ac	3.4726	1.796	0.00667	Agtr1a	4.0530	2.019	0.000638	Nppa	7.4850	2.904	0.000004540
Sbf2	3.3987	1.765	0.01505	Nr2f1	4.0250	-2.009	0.003610	Pik3c2g	7.4436	2.896	0.000001790
Igfbp5	3.2648	-1.707	0.00090	Ptgr	3.7347	-1.901	0.002070	Col3a1	7.2000	-2.848	0.000126000
Clnkb	3.2021	-1.679	0.00895	Adra1d	3.7115	1.892	0.000410	Nppa	7.0323	2.814	0.000030200
Asf1b	3.1777	-1.668	0.00899	Bdh1	3.6859	1.882	0.000156	Ucp3	6.8828	2.783	0.000003430
Kbtbd3	3.1711	-1.665	0.01990	Fam96b	3.6833	-1.881	0.000088	Ptgr	6.8116	2.768	0.000022000
Dync1li2	3.1624	1.661	0.00356	Kcne1	3.6833	1.881	0.003550	Adra1d	6.7693	-2.759	0.000572000

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Chad	3.1037	1.634	0.00262	Myl1	3.6503	1.868	0.007900	Pdp2	6.4442	-2.688	0.000003990
Spt1	3.1016	1.633	0.01112	Nalcn	3.5382	1.823	0.008440	Cd74	5.7438	-2.522	0.000119000
Aadat	3.0589	-1.613	0.00702	Hmgcr	3.5113	1.812	0.003730	Acsf6	5.6923	-2.509	0.000172000
Mmp16	3.0483	-1.608	0.01518	Penk	3.5016	-1.808	0.000279	Nr4a1	5.6256	-2.492	0.000132000
Krt19	3.0420	1.605	0.00771	Ckb	3.3104	1.727	0.000615	Cd74	5.4755	-2.453	0.000013600
Smarca2	3.0420	-1.605	0.01903	Jak3	3.2716	-1.710	0.000295	Kcnd3	5.2890	-2.403	0.000269000
Shbg	2.9752	-1.573	0.01231	Cyp2e1	3.2535	-1.702	0.005560	Higd1a	5.2561	-2.394	0.000034800
Tspy1	2.9649	1.568	0.01878	Cyp2c11	3.1755	-1.667	0.007660	Hsph1	5.2525	-2.393	0.000167000
Hbe2/Hbg1	2.8501	1.511	0.01923	Decr1	3.1210	-1.642	0.000047	Cyp2e1	5.1982	2.378	0.000050400
Cat	2.8324	1.502	0.01006	Myh7	3.0483	-1.608	0.000599	Ckb	5.0771	-2.344	0.000071900
Dbh	2.8304	1.501	0.01998	Acot2	3.0314	-1.600	0.000316	Nppa	4.9348	2.303	0.000030600
Idh1	2.7818	1.476	0.00075	Plcb2	3.0314	1.600	0.001680	Bdh1	4.8099	-2.266	0.000040600
Obp3	2.7779	1.474	0.00517	Mmp16	3.0147	-1.592	0.003840	Cckar	4.7999	2.263	0.000004480
Dio1	2.7721	1.471	0.00312	Asf1b	2.9526	1.562	0.015200	Tmem171	4.7800	2.257	0.000024700
Ascl2	2.7683	1.469	0.00531	Ppp2r2b	2.9322	1.552	0.011300	Klk1	4.6946	-2.231	0.000076600
Txn14b	2.7094	1.438	0.00456	Cited2	2.8959	-1.534	0.010000	Cyp2e1	4.6784	2.226	0.000285000
Sult1a1	2.6963	1.431	0.00232	Pgd/8kif1b	2.8779	-1.525	0.006880	Mx2	4.5789	-2.195	0.000195000
Top2a	2.6519	-1.407	0.00294	Inhbb	2.8540	1.513	0.001360	Col15a1	4.5631	-2.190	0.000002720
Tfrc	2.6244	-1.392	0.01610	Cdk18	2.8461	1.509	0.000729	Aldoc	4.5284	2.179	0.000426000
Cfd	2.6099	1.384	0.01819	Fbxo21	2.8382	-1.505	0.007990	Cd74	4.5096	-2.173	0.000022100
Bdh1	2.6027	-1.380	0.01944	Ccl3	2.8031	1.487	0.013200	Dcl1	4.4816	-2.164	0.000697000
Ace	2.5633	-1.358	0.01129	Ckb	2.7625	1.466	0.001700	Cbr1	4.4260	2.146	0.000623000
Ckb	2.5580	-1.355	0.00266	Acot2/Acot1	2.7530	-1.461	0.000435	Ucp3	4.3711	2.128	0.000119000
Pdp2	2.5245	-1.336	0.00028	Cyp2f4	2.7454	1.457	0.015200	Col1a2	4.2841	-2.099	0.000055500
Eno3	2.5036	-1.324	0.01080	G0s2	2.7378	-1.453	0.001380	Kcnq1	4.2164	-2.076	0.000262000
Pdk4	2.4880	1.315	0.00053	Olr1687	2.7264	-1.447	0.000879	Hsph1	4.1612	-2.057	0.000034900
Ivns1abp	2.4538	1.295	0.00299	Pdk4	2.6963	-1.431	0.001600	Nr4a1	4.1468	-2.052	0.000022800
Jdp2	2.4538	1.295	0.00682	Acot2	2.6945	-1.430	0.002230	Entpd2	4.1468	-2.052	0.000099300
Vipr2	2.4217	1.276	0.01533	Gpc3	2.6907	-1.428	0.005410	Acot2	4.0418	2.015	0.000005900
Chrn4	2.3768	-1.249	0.00157	Pc	2.6833	1.424	0.001740	Col1a1	4.0139	-2.005	0.000143000
Ecl1	2.3359	-1.224	0.00328	Cybrd1	2.6647	-1.414	0.003530	Rt1dmb	3.9917	-1.997	0.000126000
Alox15	2.3166	1.212	0.00376	Aldoc	2.6647	-1.414	0.003550	Ntsr2	3.9231	-1.972	0.000220000
Krtap15	2.3118	-1.209	0.00114	Spink3	2.6463	1.404	0.003970	Slc2a1	3.8852	-1.958	0.000044700
Pgr	2.3086	-1.207	0.01783	Pdlm5	2.6372	-1.399	0.000054	Acot2	3.8211	1.934	0.000004130
Sult1a1	2.2990	1.201	0.00277	Pfkfb3	2.6372	-1.399	0.004420	Mgst1	3.7633	1.912	0.000003530
Decr1	2.2942	1.198	0.00071	Serpini2	2.6281	1.394	0.003560	Igfbp3	3.7347	1.901	0.000054800
Lphn3	2.2847	1.192	0.01906	Fshb	2.5991	1.378	0.006140	Rt1da	3.6528	-1.869	0.000549000
Cyss	2.2486	1.169	0.01124	Proc	2.5901	1.373	0.010700	Gstm5	3.6075	1.851	0.000004830
Lin7a	2.2423	1.165	0.01667	Ntsr2	2.5651	1.359	0.061900	Col3a1	3.6025	-1.849	0.000026800
Fdft1	2.2115	1.145	0.00040	Impa2	2.5580	-1.355	0.000296	Bhlhe41	3.5529	-1.829	0.000012300
Ache	2.2099	1.144	0.01281	Sctr	2.5527	1.352	0.000691	Arhgap1	3.5455	1.826	0.000132000
Ephx1	2.2084	1.143	0.00019	Hpd	2.5509	1.351	0.002100	Gpc3	3.5210	1.816	0.000290000
Fbxo21	2.1795	1.124	0.00455	Thy1	2.5298	1.339	0.011300	Slc2a4	3.5186	-1.815	0.000026800
Racgap1	2.1660	-1.115	0.00361	Gclc	2.5263	-1.337	0.008750	Scd	3.4629	-1.792	0.000018800
Casq1	2.1600	-1.111	0.00010	Decr1	2.5123	-1.329	0.000578	Rt1db1	3.4534	-1.788	0.000002920
Unc13c	2.1555	-1.108	0.01759	Atp2b2	2.5105	1.328	0.002400	Agtr1a	3.4486	1.786	0.000000860
Alox15	2.1332	1.093	0.00052	Nrgn	2.5001	-1.322	0.013100	Col1a1	3.3473	-1.743	0.000178000
Cbr1	2.1199	1.084	0.00235	Nolc1	2.4949	1.319	0.007160	Pla2g2a	3.3288	1.735	0.000089900
Mmadhc	2.1067	-1.075	0.00351	Bcat2	2.4915	1.317	0.000104	Scd	3.2535	-1.702	0.000216000
Hpn	2.1067	-1.075	0.01111	Amhr2	2.4880	1.315	0.011600	Col1a1	3.2513	-1.701	0.000119000
Spock2	2.1009	1.071	0.00407	Foxq1	2.4811	1.311	0.007980	Crybb1	3.2310	-1.692	0.000679000
Fam111a	2.0994	-1.070	0.01692	Figf	2.4777	-1.309	0.001470	Dnajb1	3.2109	-1.683	0.000164000
Alb	2.0965	1.068	0.00293	Hsd17b4	2.4657	-1.302	0.009540	Lum	3.1821	-1.670	0.000010500

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Anxa6	2.0907	-1.064	0.00437	Ephx2	2.3522	-1.234	0.013500	Decr1	3.1362	1.649	0.000025200
Inha	2.0849	-1.060	0.00159	Timeless	2.3440	1.229	0.013900	Gstm5	3.1318	1.647	0.000015900
Pla2g2a	2.0705	1.05	0.00576	Slc2a1	2.3359	1.224	0.000744	Txnip	3.0674	1.617	0.000064400
Cnih2	2.0720	1.051	0.00531	Nppa	2.3166	-1.212	0.012100	Eno3	3.0293	-1.599	0.000058700
Prf1	2.0619	1.044	0.01278	Dnph1	2.3118	-1.209	0.003270	Col1a1	2.9980	-1.584	0.000446000
Ucp3	2.0449	1.032	0.00340	Gadd45a	2.2958	1.199	0.003560	Igfbp3	2.9876	1.579	0.000405000
				Txn14b	2.2942	-1.198	0.000910	Ckb	2.9180	-1.545	0.000065300
				Ucp3	2.2910	-1.196	0.003880	Sult1a1	2.9120	1.542	0.000097000
				Pbsn	2.2799	1.189	0.013800	Pfkf	2.8899	1.531	0.000094400
				Ugt1a1	2.2454	-1.167	0.000501	Pdp2	2.8540	-1.513	0.000011600
				Olr1	2.2191	-1.150	0.012300	Pcsk6	2.8461	-1.509	0.000116000
				Aqp7	2.2069	-1.142	0.000621	Aqp1	2.7972	-1.484	0.000034900
				Fcer1a	2.1856	1.128	0.001910	Entpd2	2.7837	-1.477	0.000012900
				Ech1	2.1750	-1.121	0.000966	Pak3	2.7492	1.459	0.000382000
				Ache	2.1750	1.121	0.012200	Pxmp2	2.7038	-1.435	0.000003700
				Cd36	2.1435	-1.100	0.005400	Ctsk	2.6759	-1.420	0.000085500
				P2ry1	2.1184	1.083	0.006230	Man2a1	2.6740	-1.419	0.000573000
				Pxmp2	2.1170	1.082	0.000295	Vamp5	2.6317	1.396	0.000026000
				Setd1b	2.1126	-1.079	0.010000	Cd36	2.6172	1.388	0.000039900
				Slc40a1	2.1082	-1.076	0.001720	Scn1a	2.5740	1.364	0.000454000
				Acot7	2.0936	-1.066	0.005670	Ngfrap1	2.5651	1.359	0.000164000
				Slc2a4	2.0907	1.064	0.000907	Dnajb1	2.5385	-1.344	0.000096100
				Clm3	2.0864	1.061	0.004410	Gstt2	2.5368	1.343	0.000521000
				Hk2	2.0835	1.059	0.000211	Cebpd	2.5245	1.336	0.000566000
				Txnip	2.0835	-1.059	0.000479	Chordc1	2.5175	-1.332	0.000143000
				Pla2g2a	2.0634	-1.045	0.000710	Fstl1	2.5105	-1.328	0.000134000
				Kcna4	2.0520	-1.037	0.003230	H19	2.5019	1.323	0.000441000
				Aqp1	2.0406	1.029	0.001390	Slc6a6	2.4915	1.317	0.000253000
				Chrna1	2.0350	1.025	0.015200	Bdnf	2.4743	1.307	0.000579000
				Scd	2.0335	1.024	0.002490	Dnaja1	2.4674	-1.303	0.000373000
				Hmox1	2.0293	1.021	0.009060	Adh1	2.4572	-1.297	0.000243000
				Pdp2	2.0153	1.011	0.0007697	Decr1	2.4083	1.268	0.000029000
								Sparc	2.4033	-1.265	0.000222000
								Cyp1a1	2.4016	1.264	0.000652000
								Slc3a2	2.3999	1.263	0.000563000
								Slc41a3	2.3983	1.262	0.000111000
								Pcsk6	2.3933	-1.259	0.000036600
								Cyp11a1	2.3834	-1.253	0.000012000
								Pc	2.3424	-1.228	0.000410000
								Tcap	2.3359	-1.224	0.000193000
								Ros1	2.3054	-1.205	0.000178000
								Slc3a2	2.2958	1.199	0.000441000
								Cd36	2.2894	1.195	0.000101000
								Ddit3	2.2815	-1.190	0.000436000
								Ccnd1	2.2768	-1.187	0.000107000
								Sparc	2.2752	-1.186	0.000146000
								Maoa	2.2721	-1.184	0.000511000
								Echs1	2.2517	-1.171	0.000345000
								Nr1d2	2.2454	-1.167	0.000402000
								Sparc	2.2268	-1.155	0.000104000
								Ivd	2.2038	-1.140	0.000013700
								Txnip	2.1977	1.136	0.000267000
								Ppap2a	2.1780	1.123	0.000330000

							Gstp1	2.1406	1.098	0.000785000
							Klf10	2.1391	1.097	0.000481000
							Alox15	2.1170	1.082	0.000590000
							Rab11b	2.1155	1.081	0.000334000
							Slc2a4	2.1111	-1.078	0.000571000
							Ndufa4l2	2.0994	-1.070	0.000727000
							Ptprs	2.0980	1.069	0.000164000
							Aqp7	2.0965	1.068	0.000030400
							Eno2	2.0922	1.065	0.000100000
							Myc	2.0878	1.062	0.000344000
							Insig1	2.0878	1.062	0.000362000
							Aldh1a1	2.0835	-1.059	0.000169000
							Sult1a1	2.0835	1.059	0.000607000
							Gstm1	2.0748	1.053	0.000050700
							Tns3	2.0720	1.051	0.000017700
							Ptpn3	2.0634	-1.045	0.000757000
							Lamb2	2.0520	1.037	0.000259000
							Cst3	2.0392	1.028	0.000022500
							Gstm1	2.0378	1.027	0.000068100
							Dbi	2.0111	1.008	0.000022300
							Hspb1	2.0069	-1.005	0.000329000

rno-miR-7a, rno-miR-532, rno-miR-21, rno-miR-200c, rno-miR-17-3p, rno-miR-7a*, rno-miR-186, rno-miR-203, rno-miR-322, rno-miR-187, rno-miR-92b, rno-miR-148b, rno-miR-466c, rno-miR-20b, rno-miR-9*, rno-miR-188 and rno-miR-199a compared to control rats (Table 4).

4.2. DCM-related differentially expressed mRNAs

The DCM-related gene analysis by the IPA cardiac function tool showed that seven differentially expressed mRNAs (GSE4745 dataset) were associated with cardiotoxicity (Table 1 and Table 5). *Hk2* gene was associated with cardiac necrosis and cell death, at 3, 28 and 42 days after STZ-induced diabetes. *Nppa* and *Txnip* at 28 and 42 days, and *Fstl1* and *Hspb1* at 42 days, were also associated with cardiac necrosis and cell death by different mechanisms. The *Pla2g2a* gene was related to cardiac fibrosis at all periods studied, whereas *Agtr1* and *Nppa* were associated with cardiac hypertrophy, after 28 and 42 days of the STZ treatment.

Regarding the fold regulation of the DCM-related mRNAs at day 3 after STZ-induction, the *Pla2g2a* was up-regulated (LogFC 1.0.5) and *Hk2* down-regulated (LogFC -1.8.82); at day 28, *Hk2* and *Agtr1a* were up-regulated (LogFC 2.1.53 and 2.0.19, respectively) and *Pla2g2a* and *Nppa* were down-regulated (LogFC -1.0.45 and -1.2.12, respectively); and at day 42, *Pla2g2a*, *Agtr1a*, *Nppa* and *Txnip* were up-regulated (LogFC 1.7.35, 1.7.86, 2.9.04 and 1.6.17, respectively) *Hk2*, *Fstl1* and *Hspb1* were down-regulated (LogFC -4.1.59, -1.3.28 and -1.0.05, respectively).

4.3. Regulatory miRNAs of DCM-related mRNAs

The TargetScan 7.1. web dataset predicted regulatory miRNAs of the DCM-related mRNAs from the previous analysis of the GSE4745 dataset. miRNAs target *Pla2g2a* (23 miRNAs), *Hk2* (175 miRNAs), *Agtr1* (78 miRNAs), *Nppa* (22 miRNAs), *Fstl1* (196 miRNAs), *Hspb1* (11 miRNAs) and *Txnip* (134 miRNAs) (Table 6).

4.4. mRNA-miRNAs Integrative Analysis

The Venn diagram was performed to evaluate the relationship of miRNAs found differentially expressed in the GSE44179 and the regulatory miRNAs of DCM-related mRNAs differentially expressed in the periods evaluated in GSE4745 dataset (Figure 2). Interestingly, ten miRNAs (rno-miR-214, rno-miR-320, rno-miR-877, rno-miR-34a, rno-miR-532, rno-miR-21, rno-miR-17-3p, rno-miR-322, rno-miR-187 and rno-miR-188) were differently expressed in the GSE44179 data (Table 4) and were associated with the DCM-related mRNAs in all periods evaluated, as shown in Table 2.

The *Hk2* was down-regulated at 3 and 42 days after the STZ-induction (logFC -1.8.82 and -4.1.59, respectively). It mRNA could be regulated by rno-miR-34a (LogFC 2.6.06), rno-miR-532 (LogFC 2.1.49), rno-miR-21 (LogFC 2.1.33), rno-miR-17-3p (LogFC 1.6.47), rno-miR-322 (LogFC 1.4.33), rno-miR-187 (LogFC 1.3.55), rno-miR-188 (LogFC 1.0.91), which are up-regulated in LV of diabetes-induced rats. Conversely, *Pla2g2a*, was up-regulated at 3 and 42 days (logFC 1.0.5 and 1.7.35, respectively) after diabetes-induction

Table 4. Differentially expressed miRNAs list in left ventricle of STZ-induced diabetic rats.

miRNAs	FC	logFC	p-value
rno-mir-122	17.81475	-4.16	0.00946
rno-mir-34a	6.088134	2.606	0.00424
rno-miR-7a	4.559893	2.189	0.00378
rno-mir-532	4.435203	2.149	0.04686
rno-mir-21	4.386286	2.133	0.00009
rno-mir-184	4.260433	-2.09	0.03708
rno-mir-200c	4.027822	2.01	0.00390
rno-mir-151	3.317278	-1.73	0.02562
rno-miR-17-3p	3.131817	1.647	0.01334
rno-miR-7a*	3.088701	1.627	0.01492
rno-mir-186	2.751084	1.46	0.02763
rno-mir-203	2.741566	1.455	0.00089
rno-mir-322	2.700076	1.433	0.00451
rno-mir-187	2.557971	1.355	0.02161
rno-miR-194	2.489748	-1.32	0.03604
rno-mir-22	2.479415	-1.31	0.03703
rno-mir-92b	2.472551	1.306	0.01080
rno-mir-148b	2.399943	1.263	0.00289
rno-mir-214	2.381714	-1.25	0.01773
rno-miR-30-c2*	2.268911	-1.18	0.00497
rno-mir-320	2.267339	-1.18	0.02567
rno-mir-466c	2.233026	1.159	0.00071
rno-mir-204	2.222217	-1.15	0.02982
rno-mir-20b	2.185555	1.128	0.03483
rno-miR-9*	2.131693	1.092	0.03587
rno-mir-188	2.130216	1.091	0.04111
rno-mir-342	2.116969	-1.08	0.01603
rno-mir-199a	2.108183	1.076	0.01991
rno-mir-877	2.012516	-1.01	0.02886

Table 5. DCM-related mRNAs differentially expressed in ventricle of STZ-induced diabetic rats

Period	mRNA	FC	logFC	p-value
Day 3	<i>Hk2</i>	3.6859	-1.882	0.003890
	<i>Pla2g2a</i>	2.0705	1.05	0.005760
Day 28	<i>Hk2</i>	4.4475	2.153	0.000006
	<i>Pla2g2a</i>	2.0634	-1.045	0.000710
	<i>Agtr1a</i>	4.0530	2.019	0.000640
	<i>Nppa</i>	2.3166	-1.212	0.012100
Day 42	<i>Hk2</i>	17.8642	-4.159	0.000044
	<i>Pla2g2a</i>	3.3288	1.735	0.000090
	<i>Nppa</i>	7.4850	2.904	0.000004
	<i>Fstl1</i>	2.5105	-1,328	0.000134
	<i>Hspb1</i>	2.0069	-1.005	0.000329
	<i>Txnip</i>	3.0674	1.617	0.000064

Table 6. DCM-related mRNAs differentially expressed in left ventricle 3, 28 and 42 days after STZ-induction and their regulatory miRNAs

mRNAs Target	miRNAs	Context++Score	Conservation
Agtr1	rno-miR-760-3p	-0.48	Poorly conserved sites
	rno-miR-652-5p	-0.46	Poorly conserved sites
	rno-miR-219a-5p	-0.45	Poorly conserved sites
	rno-miR-711	-0.44	Poorly conserved sites
	rno-miR-96-5p	-0.4	Poorly conserved sites
	rno-miR-547-5p	-0.33	Poorly conserved sites
	rno-miR-881-3p	-0.33	Poorly conserved sites
	rno-miR-7a-1-3p	-0.3	Poorly conserved sites
	rno-miR-1306-5p	-0.29	Poorly conserved sites
	rno-miR-873-5p	-0.28	Poorly conserved sites
	rno-miR-466b-2-3p	-0.28	Poorly conserved sites
	rno-miR-466b-4-3p	-0.28	Poorly conserved sites
	rno-miR-466b-2-3p	-0.27	Poorly conserved sites
	rno-miR-466b-4-3p	-0.27	Poorly conserved sites
	rno-miR-7a-5p	-0.26	Poorly conserved sites
	rno-miR-7b	-0.26	Poorly conserved sites
	rno-miR-3065-5p	-0.25	Poorly conserved sites
	rno-miR-194-5p	-0.25	Poorly conserved sites
	rno-miR-3085	-0.25	Poorly conserved sites
	rno-miR-29b-1-5p	-0.25	Poorly conserved sites
	rno-miR-93-3p	-0.23	Poorly conserved sites
	rno-miR-3558-3p	-0.23	Poorly conserved sites
	rno-miR-3587	-0.22	Poorly conserved sites
	rno-miR-188-3p	-0.22	Poorly conserved sites
	rno-miR-216a-3p	-0.21	Poorly conserved sites
	rno-miR-139-3p	-0.21	Poorly conserved sites
	rno-miR-344b-5p	-0.21	Poorly conserved sites
	rno-miR-29b-1-5p	-0.21	Poorly conserved sites
	rno-miR-215	-0.2	Poorly conserved sites
	rno-miR-673-5p	-0.2	Poorly conserved sites
	rno-miR-221-3p	-0.2	Poorly conserved sites
	rno-miR-222-3p	-0.2	Poorly conserved sites
	rno-miR-192-5p	-0.19	Poorly conserved sites
	rno-miR-344g	-0.19	Poorly conserved sites
	rno-miR-139-5p	-0.19	Poorly conserved sites
	rno-miR-377-3p	-0.19	Poorly conserved sites
	rno-miR-466b-4-3p	-0.19	Poorly conserved sites
	rno-miR-466b-2-3p	-0.19	Poorly conserved sites
	rno-miR-466b-2-3p	-0.19	Poorly conserved sites
	rno-miR-466b-4-3p	-0.19	Poorly conserved sites
	rno-miR-3547	-0.18	Poorly conserved sites
	rno-miR-224-3p	-0.18	Poorly conserved sites
	rno-miR-653-5p	-0.18	Poorly conserved sites

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Agtr1	rno-miR-208a-3p	-0.18	Poorly conserved sites
	rno-miR-208b-3p	-0.18	Poorly conserved sites
	rno-miR-344b-5p	-0.17	Poorly conserved sites
	rno-miR-344g	-0.17	Poorly conserved sites
	rno-miR-3593-3p	-0.17	Poorly conserved sites
	rno-miR-182	-0.17	Poorly conserved sites
	rno-miR-452-5p	-0.17	Poorly conserved sites
	rno-miR-383-3p	-0.17	Poorly conserved sites
	rno-miR-592	-0.16	Poorly conserved sites
	rno-miR-3596d	-0.16	Poorly conserved sites
	rno-miR-98-3p	-0.16	Poorly conserved sites
	rno-let-7b-3p	-0.15	Poorly conserved sites
	rno-miR-3596d	-0.15	Poorly conserved sites
	rno-let-7c-2-3p	-0.15	Poorly conserved sites
	rno-let-7f-1-3p	-0.15	Poorly conserved sites
	rno-let-7a-1-3p	-0.15	Poorly conserved sites
	rno-miR-98-3p	-0.15	Poorly conserved sites
	rno-miR-3575	-0.14	Poorly conserved sites
	rno-miR-511-3p	-0.14	Poorly conserved sites
	rno-miR-101a-5p	-0.14	Poorly conserved sites
	rno-miR-544-3p	-0.14	Poorly conserved sites
	rno-miR-499-5p	-0.14	Poorly conserved sites
	rno-miR-26b-3p	-0.14	Poorly conserved sites
	rno-let-7c-2-3p	-0.14	Poorly conserved sites
	rno-let-7f-1-3p	-0.14	Poorly conserved sites
	rno-let-7a-1-3p	-0.14	Poorly conserved sites
	rno-miR-466c-3p	-0.14	Poorly conserved sites
	rno-miR-139-5p	-0.13	Poorly conserved sites
	rno-let-7f-2-3p	-0.13	Poorly conserved sites
	rno-let-7b-3p	-0.13	Poorly conserved sites
	rno-miR-466c-3p	-0.13	Poorly conserved sites
	rno-miR-330-3p	-0.12	Poorly conserved sites
	rno-miR-466c-3p	-0.12	Poorly conserved sites
	rno-let-7f-2-3p	-0.12	Poorly conserved sites
	rno-miR-149-5p	-0.11	Poorly conserved sites
	rno-miR-9a-3p	-0.11	Poorly conserved sites
	rno-miR-350	-0.11	Poorly conserved sites
	rno-miR-204-3p	-0.11	Poorly conserved sites
	rno-miR-466c-3p	-0.11	Poorly conserved sites
	rno-miR-466b-2-3p	-0.11	Poorly conserved sites
	rno-miR-466b-4-3p	-0.11	Poorly conserved sites
	rno-miR-208b-5p	-0.1	Poorly conserved sites
	rno-miR-466b-2-3p	-0.1	Poorly conserved sites
	rno-miR-466b-4-3p	-0.1	Poorly conserved sites
	rno-miR-3085	-0.09	Poorly conserved sites
	rno-miR-674-5p	-0.09	Poorly conserved sites
	rno-miR-196c-3p	-0.09	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

Agtr1	rno-miR-881-5p	-0.09	Poorly conserved sites
	rno-miR-466c-3p	-0.09	Poorly conserved sites
	rno-miR-871-5p	-0.08	Poorly conserved sites
	rno-miR-3585-5p	-0.08	Poorly conserved sites
	rno-miR-881-5p	-0.08	Poorly conserved sites
	rno-miR-743a-5p	-0.08	Poorly conserved sites
	rno-miR-466c-3p	-0.08	Poorly conserved sites
	rno-miR-743a-5p	-0.07	Poorly conserved sites
	rno-miR-323-3p	-0.07	Poorly conserved sites
	rno-miR-26b-5p	-0.07	Poorly conserved sites
	rno-miR-871-5p	-0.07	Poorly conserved sites
	rno-miR-208a-5p	-0.06	Poorly conserved sites
	rno-miR-26a-5p	-0.06	Poorly conserved sites
	rno-miR-144-3p	-0.06	Poorly conserved sites
	rno-miR-471-3p	-0.04	Poorly conserved sites
	rno-miR-101b-3p	-0.04	Poorly conserved sites
	rno-miR-101a-3p	-0.04	Poorly conserved sites
	rno-miR-488-3p	-0.03	Poorly conserved sites
	rno-miR-873-5p	-0.02	Poorly conserved sites
	rno-miR-132-3p	-0.02	Poorly conserved sites
	rno-miR-551b-5p	-0.02	Poorly conserved sites
	rno-miR-543-3p	-0.02	Poorly conserved sites
	rno-miR-212-3p	-0.01	Poorly conserved sites
	rno-miR-32-3p	-0.01	Poorly conserved sites
	rno-miR-19b-1-5p	-0.01	Poorly conserved sites
	rno-miR-19b-2-5p	-0.01	Poorly conserved sites
	rno-miR-3551-3p	-0.01	Poorly conserved sites
	rno-miR-3551-3p	-0.01	Poorly conserved sites
	rno-miR-3551-3p	-0.01	Poorly conserved sites
	rno-miR-410-3p	-0.01	Poorly conserved sites
	rno-miR-344b-1-3p	-0.01	Poorly conserved sites
Fstl1	rno-miR-31b	N/A	Poorly conserved sites
	rno-miR-31b	N/A	Poorly conserved sites
	rno-miR-598-5p	-0.62	Poorly conserved sites
	rno-miR-1247-5p	-0.54	Poorly conserved sites
	rno-miR-340-3p	-0.5	Poorly conserved sites
	rno-miR-23a-5p	-0.48	Poorly conserved sites
	rno-miR-337-5p	-0.46	Poorly conserved sites
	rno-miR-874-5p	-0.43	Poorly conserved sites
	rno-miR-6328	-0.41	Poorly conserved sites
	rno-miR-3590-5p	-0.37	Poorly conserved sites
	rno-miR-29b-3p	-0.35	Conserved sites
	rno-miR-29c-3p	-0.35	Conserved sites
	rno-miR-29a-3p	-0.35	Conserved sites
	rno-miR-652-3p	-0.35	Poorly conserved sites
	rno-miR-666-3p	-0.3	Poorly conserved sites
	rno-miR-9a-5p	-0.29	Conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

Fstl1	rno-miR-31a-3p	-0.29	Poorly conserved sites
	rno-miR-540-5p	-0.28	Poorly conserved sites
	rno-miR-191a-5p	-0.28	Poorly conserved sites
	mo-miR-1193-5p	-0.27	Poorly conserved sites
	mo-miR-203a-5p	-0.27	Poorly conserved sites
	rno-miR-218a-2-3p	-0.27	Poorly conserved sites
	rno-miR-208b-5p	-0.27	Poorly conserved sites
	rno-miR-218a-5p	-0.27	Poorly conserved sites
	mo-miR-504	-0.26	Poorly conserved sites
	rno-miR-3557-3p	-0.26	Poorly conserved sites
	rno-miR-872-5p	-0.26	Poorly conserved sites
	rno-miR-29c-5p	-0.26	Poorly conserved sites
	mo-miR-124-3p	-0.25	Conserved sites
	mo-miR-667-5p	-0.25	Poorly conserved sites
	rno-miR-582-3p	-0.25	Poorly conserved sites
	rno-miR-137-3p	-0.24	Conserved sites
	rno-miR-3064-3p	-0.24	Poorly conserved sites
	mo-miR-345-5p	-0.24	Poorly conserved sites
	mo-miR-346	-0.24	Poorly conserved sites
	rno-miR-378a-3p	-0.23	Conserved sites
	rno-miR-22-3p	-0.23	Conserved sites
	mo-miR-499-3p	-0.23	Poorly conserved sites
	mo-miR-322-3p	-0.23	Poorly conserved sites
	rno-miR-200b-3p	-0.22	Conserved sites
	rno-miR-200c-3p	-0.22	Conserved sites
	rno-miR-18a-5p	-0.22	Poorly conserved sites
	mo-miR-30b-3p	-0.22	Poorly conserved sites
	mo-miR-412-3p	-0.22	Poorly conserved sites
	rno-miR-342-5p	-0.22	Poorly conserved sites
	rno-miR-429	-0.21	Conserved sites
	rno-miR-206-5p	-0.21	Poorly conserved sites
	mo-miR-216a-3p	-0.21	Poorly conserved sites
	rno-miR-10a-5p	-0.21	Poorly conserved sites
	rno-miR-208a-5p	-0.21	Poorly conserved sites
	rno-miR-540-5p	-0.2	Poorly conserved sites
	mo-miR-451-3p	-0.2	Poorly conserved sites
	mo-miR-497-3p	-0.2	Poorly conserved sites
	rno-miR-667-5p	-0.2	Poorly conserved sites
	rno-miR-384-3p	-0.19	Poorly conserved sites
	rno-miR-224-3p	-0.19	Poorly conserved sites
	mo-miR-99a-3p	-0.19	Poorly conserved sites
	mo-miR-99b-3p	-0.19	Poorly conserved sites
	rno-miR-452-3p	-0.19	Poorly conserved sites
	rno-miR-363-3p	-0.18	Conserved sites
	rno-miR-134-5p	-0.18	Poorly conserved sites
	mo-miR-3564	-0.18	Poorly conserved sites
	rno-miR-378a-3p	-0.18	Poorly conserved sites
	rno-miR-92a-2-5p	-0.18	Poorly conserved sites
	rno-miR-615	-0.18	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

Fstl1	rno-miR-743b-5p	-0.18	Poorly conserved sites
	rno-miR-3558-3p	-0.18	Poorly conserved sites
	rno-miR-540-3p	-0.18	Poorly conserved sites
	rno-miR-598-3p	-0.18	Poorly conserved sites
	rno-miR-5132-5p	-0.17	Poorly conserved sites
	rno-miR-298-3p	-0.17	Poorly conserved sites
	rno-miR-327	-0.17	Poorly conserved sites
	rno-miR-24-3p	-0.17	Poorly conserved sites
	rno-miR-218a-5p	-0.17	Poorly conserved sites
	rno-miR-880-3p	-0.17	Poorly conserved sites
	rno-miR-32-5p	-0.16	Conserved sites
	rno-miR-673-5p	-0.16	Poorly conserved sites
	rno-miR-449a-3p	-0.16	Poorly conserved sites
	rno-miR-499-5p	-0.16	Poorly conserved sites
	rno-miR-741-3p	-0.16	Poorly conserved sites
	rno-miR-24-3p	-0.16	Poorly conserved sites
	rno-miR-3541	-0.16	Poorly conserved sites
	rno-miR-1297	-0.16	Poorly conserved sites
	rno-miR-652-5p	-0.16	Poorly conserved sites
	rno-miR-138-5p	-0.16	Poorly conserved sites
	rno-miR-488-5p	-0.15	Poorly conserved sites
	rno-miR-15a-5p	-0.15	Poorly conserved sites
	rno-miR-15b-5p	-0.15	Poorly conserved sites
	rno-miR-125a-3p	-0.15	Poorly conserved sites
	rno-miR-547-5p	-0.15	Poorly conserved sites
	rno-miR-378a-5p	-0.15	Poorly conserved sites
	rno-miR-877	-0.15	Poorly conserved sites
	rno-miR-328a-3p	-0.15	Poorly conserved sites
	rno-miR-328b-3p	-0.15	Poorly conserved sites
	rno-miR-299a-3p	-0.15	Poorly conserved sites
	rno-miR-299b-3p	-0.15	Poorly conserved sites
	rno-miR-6323	-0.15	Poorly conserved sites
	rno-miR-3594-5p	-0.15	Poorly conserved sites
	rno-miR-540-5p	-0.15	Poorly conserved sites
	rno-miR-218a-5p	-0.15	Poorly conserved sites
	rno-miR-190b-5p	-0.15	Poorly conserved sites
	rno-miR-497-5p	-0.14	Poorly conserved sites
	rno-miR-16-5p	-0.14	Poorly conserved sites
	rno-miR-204-3p	-0.14	Poorly conserved sites
	rno-miR-218a-5p	-0.14	Poorly conserved sites
	rno-miR-216a-5p	-0.14	Poorly conserved sites
	rno-miR-15a-5p	-0.14	Poorly conserved sites
	rno-miR-195-5p	-0.14	Poorly conserved sites
	rno-miR-15b-5p	-0.14	Poorly conserved sites
	rno-miR-140-3p	-0.14	Poorly conserved sites
	rno-miR-327	-0.14	Poorly conserved sites
	rno-miR-497-3p	-0.14	Poorly conserved sites
	rno-miR-3594-5p	-0.14	Poorly conserved sites
	rno-miR-25-3p	-0.13	Conserved sites
	rno-miR-383-3p	-0.13	Poorly conserved sites
	rno-miR-322-5p	-0.13	Poorly conserved sites

Fstl1	rno-miR-363-5p	-0.13	Poorly conserved sites
	rno-miR-217-3p	-0.13	Poorly conserved sites
	rno-miR-206-3p	-0.13	Poorly conserved sites
	rno-miR-1b	-0.13	Poorly conserved sites
	rno-miR-1-3p	-0.13	Poorly conserved sites
	rno-miR-16-5p	-0.13	Poorly conserved sites
	rno-miR-433-3p	-0.13	Poorly conserved sites
	rno-miR-92a-2-5p	-0.13	Poorly conserved sites
	rno-miR-190a-5p	-0.13	Poorly conserved sites
	rno-miR-471-3p	-0.12	Poorly conserved sites
	rno-miR-350	-0.12	Poorly conserved sites
	rno-miR-486	-0.12	Poorly conserved sites
	rno-miR-7578	-0.12	Poorly conserved sites
	rno-miR-322-5p	-0.12	Poorly conserved sites
	rno-miR-497-5p	-0.12	Poorly conserved sites
	rno-miR-323-5p	-0.12	Poorly conserved sites
	rno-miR-5132-3p	-0.12	Poorly conserved sites
	rno-miR-30c-1-3p	-0.12	Poorly conserved sites
	rno-miR-3580-5p	-0.12	Poorly conserved sites
	rno-miR-92a-3p	-0.11	Conserved sites
	rno-miR-92b-3p	-0.11	Conserved sites
	rno-miR-495	-0.11	Poorly conserved sites
	rno-miR-214-3p	-0.11	Poorly conserved sites
	rno-miR-195-5p	-0.11	Poorly conserved sites
	rno-miR-6327	-0.11	Poorly conserved sites
	rno-miR-6315	-0.11	Poorly conserved sites
	rno-miR-203b-3p	-0.11	Poorly conserved sites
	rno-miR-18a-3p	-0.11	Poorly conserved sites
	rno-miR-124-5p	-0.11	Poorly conserved sites
	rno-miR-743b-5p	-0.11	Poorly conserved sites
	rno-miR-3580-5p	-0.11	Poorly conserved sites
	rno-miR-881-3p	-0.11	Poorly conserved sites
	rno-miR-30c-2-3p	-0.11	Poorly conserved sites
	rno-miR-20a-3p	-0.11	Poorly conserved sites
	rno-miR-9a-3p	-0.11	Poorly conserved sites
	rno-miR-3561-3p	-0.11	Poorly conserved sites
	rno-miR-1949	-0.11	Poorly conserved sites
	rno-miR-300-5p	-0.1	Poorly conserved sites
	rno-miR-547-5p	-0.1	Poorly conserved sites
	rno-miR-3084a-3p	-0.1	Poorly conserved sites
	rno-miR-3084b-3p	-0.1	Poorly conserved sites
	rno-miR-3084d	-0.1	Poorly conserved sites
	rno-miR-330-3p	-0.1	Poorly conserved sites
	rno-miR-497-3p	-0.1	Poorly conserved sites
	rno-miR-547-3p	-0.1	Poorly conserved sites
	rno-miR-741-3p	-0.1	Poorly conserved sites
	rno-miR-125a-3p	-0.1	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

	rno-miR-185-5p	-0.1	Poorly conserved sites
	rno-miR-18a-3p	-0.1	Poorly conserved sites
	rno-miR-221-5p	-0.1	Poorly conserved sites
	rno-miR-205	-0.1	Poorly conserved sites
Fstl1	rno-miR-1896	-0.1	Poorly conserved sites
	rno-miR-2985	-0.1	Poorly conserved sites
	rno-miR-218a-1-3p	-0.1	Poorly conserved sites
	rno-miR-675-3p	-0.09	Poorly conserved sites
	rno-miR-3589	-0.09	Poorly conserved sites
	rno-miR-6331	-0.09	Poorly conserved sites
	rno-miR-451-3p	-0.09	Poorly conserved sites
	rno-miR-6325	-0.09	Poorly conserved sites
	rno-miR-6330	-0.09	Poorly conserved sites
	rno-miR-139-5p	-0.09	Poorly conserved sites
	rno-miR-370-3p	-0.09	Poorly conserved sites
	rno-miR-370-5p	-0.09	Poorly conserved sites
	rno-miR-653-5p	-0.09	Poorly conserved sites
	rno-miR-138-5p	-0.09	Poorly conserved sites
	rno-miR-3084b-3p	-0.09	Poorly conserved sites
	rno-miR-3084d	-0.09	Poorly conserved sites
	rno-miR-3084a-3p	-0.09	Poorly conserved sites
	rno-miR-509-5p	-0.09	Poorly conserved sites
	rno-miR-9a-3p	-0.08	Poorly conserved sites
	rno-miR-1298	-0.08	Poorly conserved sites
	rno-miR-136-5p	-0.08	Poorly conserved sites
	rno-miR-452-5p	-0.08	Poorly conserved sites
	rno-miR-183-5p	-0.08	Poorly conserved sites
	rno-miR-27b-3p	-0.08	Poorly conserved sites
	rno-miR-27a-3p	-0.08	Poorly conserved sites
	rno-miR-743a-5p	-0.07	Poorly conserved sites
	rno-miR-23a-5p	-0.07	Poorly conserved sites
	rno-miR-761	-0.07	Poorly conserved sites
	rno-miR-539-5p	-0.07	Poorly conserved sites
	rno-miR-135a-5p	-0.07	Poorly conserved sites
	rno-miR-135b-5p	-0.07	Poorly conserved sites
	rno-miR-544-5p	-0.07	Poorly conserved sites
	rno-miR-349	-0.07	Poorly conserved sites
	rno-miR-1193-3p	-0.07	Poorly conserved sites
	rno-miR-452-5p	-0.07	Poorly conserved sites
	rno-miR-3553	-0.07	Poorly conserved sites
	rno-miR-33-5p	-0.07	Poorly conserved sites
	rno-miR-212-5p	-0.07	Poorly conserved sites
	rno-miR-874-3p	-0.06	Poorly conserved sites
	rno-miR-18a-3p	-0.06	Poorly conserved sites
	rno-miR-871-5p	-0.06	Poorly conserved sites
	rno-miR-3586-5p	-0.06	Poorly conserved sites
	rno-miR-339-5p	-0.06	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

	rno-miR-299b-3p	-0.06	Poorly conserved sites
	rno-miR-299a-3p	-0.06	Poorly conserved sites
	rno-miR-6323	-0.06	Poorly conserved sites
	rno-miR-497-3p	-0.06	Poorly conserved sites
	rno-miR-1193-5p	-0.06	Poorly conserved sites
	rno-miR-873-5p	-0.06	Poorly conserved sites
	rno-miR-200c-3p	-0.06	Poorly conserved sites
	rno-miR-429	-0.06	Poorly conserved sites
Fstl1	rno-miR-200b-3p	-0.06	Poorly conserved sites
	rno-miR-300-5p	-0.06	Poorly conserved sites
	rno-let-7g-3p	-0.06	Poorly conserved sites
	rno-miR-667-5p	-0.06	Poorly conserved sites
	rno-miR-338-3p	-0.06	Poorly conserved sites
	rno-miR-1193-5p	-0.06	Poorly conserved sites
	rno-miR-222-5p	-0.05	Poorly conserved sites
	rno-miR-382-3p	-0.05	Poorly conserved sites
	rno-miR-505-5p	-0.05	Poorly conserved sites
	rno-miR-3585-5p	-0.05	Poorly conserved sites
	rno-miR-6328	-0.05	Poorly conserved sites
	rno-miR-298-5p	-0.05	Poorly conserved sites
	rno-miR-3569	-0.05	Poorly conserved sites
	rno-miR-3589	-0.05	Poorly conserved sites
	rno-miR-6325	-0.05	Poorly conserved sites
	rno-miR-544-3p	-0.05	Poorly conserved sites
	rno-let-7c-1-3p	-0.05	Poorly conserved sites
	rno-miR-208a-3p	-0.05	Poorly conserved sites
	rno-miR-208b-3p	-0.05	Poorly conserved sites
	rno-miR-200a-5p	-0.05	Poorly conserved sites
	rno-miR-203a-3p	-0.04	Conserved sites
	rno-miR-214-3p	-0.04	Poorly conserved sites
	rno-miR-3561-5p	-0.04	Poorly conserved sites
	rno-miR-452-3p	-0.04	Poorly conserved sites
	rno-miR-342-3p	-0.04	Poorly conserved sites
	rno-miR-18a-3p	-0.04	Poorly conserved sites
	rno-miR-764-5p	-0.04	Poorly conserved sites
	rno-miR-759	-0.04	Poorly conserved sites
	rno-miR-495	-0.04	Poorly conserved sites
	rno-miR-3068-3p	-0.04	Poorly conserved sites
	rno-miR-3084c-5p	-0.04	Poorly conserved sites
	rno-miR-3084b-5p	-0.04	Poorly conserved sites
	rno-miR-3557-5p	-0.04	Poorly conserved sites
	rno-let-7a-2-3p	-0.04	Poorly conserved sites
	rno-miR-488-3p	-0.04	Poorly conserved sites
	rno-miR-223-5p	-0.03	Poorly conserved sites
	rno-miR-212-5p	-0.03	Poorly conserved sites
	rno-miR-1224	-0.03	Poorly conserved sites
	rno-miR-497-3p	-0.03	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

	rno-miR-499-5p	-0.03	Poorly conserved sites
	rno-miR-382-5p	-0.02	Poorly conserved sites
	rno-miR-881-5p	-0.02	Poorly conserved sites
	rno-miR-761	-0.02	Poorly conserved sites
	rno-miR-138-2-3p	-0.02	Poorly conserved sites
	rno-miR-181a-2-3p	-0.02	Poorly conserved sites
	rno-miR-23b-5p	-0.02	Poorly conserved sites
	rno-miR-143-5p	-0.02	Poorly conserved sites
	rno-miR-551b-5p	-0.02	Poorly conserved sites
	rno-miR-3589	-0.02	Poorly conserved sites
	rno-miR-1224	-0.02	Poorly conserved sites
	rno-miR-3065-5p	-0.02	Poorly conserved sites
Fstl1	rno-miR-7a-1-3p	-0.02	Poorly conserved sites
	rno-miR-19a-3p	-0.02	Poorly conserved sites
	rno-miR-19b-3p	-0.02	Poorly conserved sites
	rno-miR-330-3p	-0.02	Poorly conserved sites
	rno-miR-3583-3p	-0.02	Poorly conserved sites
	rno-miR-3560	-0.02	Poorly conserved sites
	rno-miR-875	-0.02	Poorly conserved sites
	rno-miR-873-3p	-0.02	Poorly conserved sites
	rno-miR-449c-3p	-0.02	Poorly conserved sites
	rno-miR-497-3p	-0.02	Poorly conserved sites
	rno-miR-877	-0.02	Poorly conserved sites
	rno-miR-20b-3p	-0.02	Poorly conserved sites
	rno-miR-17-1-3p	-0.02	Poorly conserved sites
	rno-miR-873-3p	-0.02	Poorly conserved sites
	rno-miR-743a-5p	-0.02	Poorly conserved sites
	rno-miR-871-5p	-0.02	Poorly conserved sites
	rno-miR-881-5p	-0.02	Poorly conserved sites
	rno-miR-126a-5p	-0.02	Poorly conserved sites
	rno-miR-290	-0.02	Poorly conserved sites
	rno-miR-292-5p	-0.02	Poorly conserved sites
	rno-miR-293-5p	-0.02	Poorly conserved sites
	rno-miR-181a-2-3p	-0.02	Poorly conserved sites
	rno-miR-488-3p	-0.02	Poorly conserved sites
	rno-miR-539-5p	-0.02	Poorly conserved sites
	rno-miR-1297	-0.02	Poorly conserved sites
	rno-miR-379-3p	-0.02	Poorly conserved sites
	rno-miR-144-3p	-0.02	Poorly conserved sites
	rno-miR-128-3p	-0.02	Poorly conserved sites
	rno-miR-216b-3p	-0.02	Poorly conserved sites
	rno-miR-325-3p	-0.02	Poorly conserved sites
	rno-miR-200b-5p	-0.02	Poorly conserved sites
	rno-miR-101a-5p	-0.02	Poorly conserved sites
	rno-miR-485-5p	-0.02	Poorly conserved sites
	rno-miR-421-5p	-0.02	Poorly conserved sites
	rno-miR-325-3p	-0.01	Conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

	rno-miR-338-3p	-0.01	Poorly conserved sites
	rno-miR-185-3p	-0.01	Poorly conserved sites
	rno-miR-873-5p	-0.01	Poorly conserved sites
	rno-miR-3588	-0.01	Poorly conserved sites
	rno-miR-129-5p	-0.01	Poorly conserved sites
	rno-miR-764-3p	-0.01	Poorly conserved sites
	rno-miR-203a-3p	-0.01	Poorly conserved sites
	rno-miR-186-5p	-0.01	Poorly conserved sites
	rno-miR-186-3p	-0.01	Poorly conserved sites
	rno-miR-495	-0.01	Poorly conserved sites
	rno-miR-300-3p	-0.01	Poorly conserved sites
	rno-miR-495	-0.01	Poorly conserved sites
	rno-miR-495	-0.01	Poorly conserved sites
	rno-miR-153-5p	-0.01	Poorly conserved sites
	rno-miR-873-5p	-0.01	Poorly conserved sites
	rno-miR-203a-3p	-0.01	Poorly conserved sites
Fstl1	rno-miR-325-3p	-0.01	Poorly conserved sites
	rno-miR-3570	-0.01	Poorly conserved sites
	rno-miR-493-5p	-0.01	Poorly conserved sites
	rno-miR-320-3p	-0.01	Poorly conserved sites
	rno-miR-411-3p	-0.01	Poorly conserved sites
	rno-miR-3065-3p	-0.01	Poorly conserved sites
	rno-miR-451-3p	-0.01	Poorly conserved sites
	rno-miR-290	-0.01	Poorly conserved sites
	rno-miR-292-5p	-0.01	Poorly conserved sites
	rno-miR-293-5p	-0.01	Poorly conserved sites
	rno-miR-295-5p	-0.01	Poorly conserved sites
Hk2	rno-miR-323-5p	-0.41	Poorly conserved sites
	rno-miR-132-5p	-0.4	Poorly conserved sites
	rno-miR-1839-3p	-0.36	Poorly conserved sites
	rno-miR-380-5p	-0.33	Poorly conserved sites
	rno-miR-9b-5p	-0.33	Poorly conserved sites
	rno-miR-3561-3p	-0.27	Poorly conserved sites
	rno-miR-615	-0.26	Poorly conserved sites
	rno-miR-423-3p	-0.24	Poorly conserved sites
	rno-miR-24-3p	-0.23	Poorly conserved sites
	rno-miR-184	-0.22	Poorly conserved sites
	rno-miR-499-3p	-0.22	Poorly conserved sites
	rno-miR-101b-5p	-0.2	Poorly conserved sites
	rno-miR-3551-5p	-0.2	Poorly conserved sites
	rno-let-7d-5p	-0.19	Conserved sites
	rno-miR-187-5p	-0.19	Poorly conserved sites
	rno-miR-17-2-3p	-0.19	Poorly conserved sites
	rno-miR-329-3p	-0.19	Poorly conserved sites
	rno-miR-125a-5p	-0.18	Conserved sites
	rno-miR-351-5p	-0.18	Conserved sites
	rno-miR-7578	-0.18	Poorly conserved sites

	rno-miR-6326	-0.18	Poorly conserved sites
	rno-miR-187-5p	-0.18	Poorly conserved sites
	rno-miR-362-3p	-0.18	Poorly conserved sites
	rno-let-7g-5p	-0.17	Conserved sites
	rno-miR-3577	-0.17	Poorly conserved sites
	rno-miR-202-3p	-0.17	Poorly conserved sites
	rno-miR-9a-5p	-0.16	Conserved sites
	rno-let-7e-5p	-0.16	Conserved sites
	rno-let-7a-5p	-0.16	Conserved sites
	rno-let-7f-5p	-0.16	Conserved sites
	rno-let-7i-5p	-0.16	Conserved sites
	rno-miR-125b-5p	-0.16	Conserved sites
	rno-miR-667-5p	-0.16	Poorly conserved sites
	rno-miR-218b	-0.16	Poorly conserved sites
	rno-miR-323-3p	-0.15	Conserved sites
	rno-miR-3541	-0.15	Poorly conserved sites
	rno-let-7b-5p	-0.14	Conserved sites
	rno-miR-98-5p	-0.14	Conserved sites
	rno-let-7c-5p	-0.14	Conserved sites
	rno-miR-10b-3p	-0.14	Poorly conserved sites
Hk2	rno-miR-1912-3p	-0.14	Poorly conserved sites
	rno-miR-3592	-0.14	Poorly conserved sites
	rno-miR-9a-5p	-0.14	Poorly conserved sites
	rno-miR-135a-5p	-0.14	Poorly conserved sites
	rno-miR-135b-5p	-0.14	Poorly conserved sites
	rno-miR-143-3p	-0.13	Conserved sites
	rno-miR-149-3p	-0.13	Poorly conserved sites
	rno-miR-219a-1-3p	-0.12	Poorly conserved sites
	rno-miR-3577	-0.12	Poorly conserved sites
	rno-miR-3559-5p	-0.12	Poorly conserved sites
	rno-miR-6334	-0.12	Poorly conserved sites
	rno-miR-139-5p	-0.11	Conserved sites
	rno-miR-3102	-0.11	Poorly conserved sites
	rno-miR-9a-5p	-0.11	Poorly conserved sites
	rno-miR-192-5p	-0.11	Poorly conserved sites
	rno-miR-215	-0.11	Poorly conserved sites
	rno-miR-6326	-0.11	Poorly conserved sites
	rno-miR-344a-5p	-0.11	Poorly conserved sites
	rno-let-7a-2-3p	-0.1	Poorly conserved sites
	rno-miR-337-3p	-0.1	Poorly conserved sites
	rno-miR-743b-5p	-0.1	Poorly conserved sites
	rno-miR-343	-0.1	Poorly conserved sites
	rno-miR-9a-5p	-0.1	Poorly conserved sites
	rno-miR-128-3p	-0.1	Poorly conserved sites
	rno-miR-324-5p	-0.1	Poorly conserved sites
	rno-miR-191a-3p	-0.1	Poorly conserved sites
	rno-miR-206-5p	-0.1	Poorly conserved sites

	rno-miR-541-5p	-0.1	Poorly conserved sites
	rno-miR-741-5p	-0.1	Poorly conserved sites
	rno-miR-376c-3p	-0.1	Poorly conserved sites
	rno-miR-218a-1-3p	-0.09	Poorly conserved sites
	rno-miR-133a-3p	-0.09	Poorly conserved sites
	rno-miR-6331	-0.09	Poorly conserved sites
	rno-miR-484	-0.09	Poorly conserved sites
	rno-miR-6321	-0.09	Poorly conserved sites
	rno-miR-347	-0.09	Poorly conserved sites
	rno-miR-3571	-0.09	Poorly conserved sites
	rno-miR-336-3p	-0.09	Poorly conserved sites
	rno-miR-343	-0.08	Poorly conserved sites
	rno-miR-344a-3p	-0.08	Poorly conserved sites
	rno-miR-3561-3p	-0.08	Poorly conserved sites
	rno-miR-133b-3p	-0.08	Poorly conserved sites
	rno-miR-138-1-3p	-0.08	Poorly conserved sites
	rno-miR-298-5p	-0.08	Poorly conserved sites
	rno-miR-140-3p	-0.08	Poorly conserved sites
	rno-miR-3084d	-0.07	Poorly conserved sites
	rno-miR-3084b-3p	-0.07	Poorly conserved sites
	rno-miR-3084a-3p	-0.07	Poorly conserved sites
	rno-let-7g-3p	-0.07	Poorly conserved sites
	rno-miR-202-3p	-0.07	Poorly conserved sites
	rno-let-7f-2-3p	-0.07	Poorly conserved sites
Hk2	rno-miR-200a-3p	-0.07	Poorly conserved sites
	rno-miR-141-3p	-0.07	Poorly conserved sites
	rno-miR-201-5p	-0.07	Poorly conserved sites
	rno-miR-141-5p	-0.06	Poorly conserved sites
	rno-miR-3557-3p	-0.06	Poorly conserved sites
	rno-miR-214-5p	-0.06	Poorly conserved sites
	rno-let-7d-5p	-0.06	Poorly conserved sites
	rno-miR-322-3p	-0.06	Poorly conserved sites
	rno-miR-760-3p	-0.06	Poorly conserved sites
	rno-miR-34a-5p	-0.06	Poorly conserved sites
	rno-miR-412-3p	-0.06	Poorly conserved sites
	rno-miR-216a-3p	-0.05	Poorly conserved sites
	rno-let-7c-1-3p	-0.05	Poorly conserved sites
	rno-miR-344g	-0.05	Poorly conserved sites
	rno-miR-3120	-0.05	Poorly conserved sites
	rno-miR-10b-3p	-0.05	Poorly conserved sites
	rno-miR-188-3p	-0.05	Poorly conserved sites
	rno-miR-448-5p	-0.05	Poorly conserved sites
	rno-miR-879-5p	-0.05	Poorly conserved sites
	rno-miR-485-5p	-0.05	Poorly conserved sites
	rno-miR-501-3p	-0.05	Poorly conserved sites
	rno-miR-500-3p	-0.05	Poorly conserved sites

	rno-miR-3084a-5p	-0.05	Poorly conserved sites
	rno-miR-140-5p	-0.05	Poorly conserved sites
	rno-miR-344b-5p	-0.04	Poorly conserved sites
	rno-miR-98-5p	-0.04	Poorly conserved sites
	rno-let-7g-5p	-0.04	Poorly conserved sites
	rno-miR-185-5p	-0.04	Poorly conserved sites
	rno-miR-3587	-0.04	Poorly conserved sites
	rno-miR-758-5p	-0.04	Poorly conserved sites
	rno-miR-3084c-5p	-0.04	Poorly conserved sites
	rno-miR-3084b-5p	-0.04	Poorly conserved sites
	rno-miR-34c-5p	-0.04	Poorly conserved sites
	rno-miR-34b-5p	-0.04	Poorly conserved sites
	rno-miR-362-3p	-0.04	Poorly conserved sites
	rno-miR-329-3p	-0.04	Poorly conserved sites
	rno-miR-295-3p	-0.04	Poorly conserved sites
	rno-miR-452-3p	-0.03	Poorly conserved sites
	rno-miR-532-3p	-0.03	Poorly conserved sites
	rno-miR-300-5p	-0.03	Poorly conserved sites
	rno-miR-742-3p	-0.03	Poorly conserved sites
	rno-let-7i-5p	-0.03	Poorly conserved sites
	rno-let-7b-5p	-0.03	Poorly conserved sites
	rno-let-7a-5p	-0.03	Poorly conserved sites
	rno-let-7c-5p	-0.03	Poorly conserved sites
	rno-let-7e-5p	-0.03	Poorly conserved sites
	rno-let-7f-5p	-0.03	Poorly conserved sites
	rno-miR-504	-0.03	Poorly conserved sites
	rno-miR-3084d	-0.03	Poorly conserved sites
	rno-miR-3084b-3p	-0.03	Poorly conserved sites
	rno-miR-3084a-3p	-0.03	Poorly conserved sites
Hk2	rno-miR-452-3p	-0.03	Poorly conserved sites
	rno-miR-135a-5p	-0.03	Poorly conserved sites
	rno-miR-135b-5p	-0.03	Poorly conserved sites
	rno-miR-6331	-0.03	Poorly conserved sites
	rno-miR-880-5p	-0.03	Poorly conserved sites
	rno-miR-32-3p	-0.03	Poorly conserved sites
	rno-miR-211-3p	-0.03	Poorly conserved sites
	rno-miR-449c-5p	-0.03	Poorly conserved sites
	rno-miR-21-5p	-0.03	Poorly conserved sites
	rno-miR-3553	-0.02	Poorly conserved sites
	rno-miR-873-5p	-0.02	Poorly conserved sites
	rno-miR-761	-0.02	Poorly conserved sites
	rno-miR-214-3p	-0.02	Poorly conserved sites
	rno-miR-665	-0.02	Poorly conserved sites
	rno-miR-5132-3p	-0.02	Poorly conserved sites
	rno-miR-33-3p	-0.02	Poorly conserved sites
	rno-miR-185-3p	-0.02	Poorly conserved sites

	rno-miR-145-3p	-0.02	Poorly conserved sites
	rno-miR-181a-5p	-0.02	Poorly conserved sites
	rno-miR-181d-5p	-0.02	Poorly conserved sites
	rno-miR-181b-5p	-0.02	Poorly conserved sites
	rno-miR-181c-5p	-0.02	Poorly conserved sites
	rno-miR-425-5p	-0.02	Poorly conserved sites
	rno-miR-6322	-0.02	Poorly conserved sites
	rno-miR-679	-0.02	Poorly conserved sites
	rno-miR-3544	-0.02	Poorly conserved sites
	rno-miR-742-3p	-0.02	Poorly conserved sites
	rno-miR-190a-3p	-0.02	Poorly conserved sites
	rno-miR-672-5p	-0.02	Poorly conserved sites
	rno-miR-377-5p	-0.02	Poorly conserved sites
	rno-miR-665	-0.02	Poorly conserved sites
	rno-miR-361-3p	-0.02	Poorly conserved sites
	rno-miR-495	-0.02	Poorly conserved sites
	rno-miR-222-5p	-0.02	Poorly conserved sites
	rno-miR-568	-0.02	Poorly conserved sites
	rno-miR-212-3p	-0.02	Poorly conserved sites
	rno-miR-132-3p	-0.02	Poorly conserved sites
	rno-miR-871-5p	-0.02	Poorly conserved sites
	rno-miR-743a-5p	-0.02	Poorly conserved sites
	rno-miR-881-5p	-0.02	Poorly conserved sites
	rno-miR-3557-5p	-0.02	Poorly conserved sites
	rno-miR-488-3p	-0.02	Poorly conserved sites
	rno-miR-3542	-0.02	Poorly conserved sites
	rno-miR-411-3p	-0.02	Poorly conserved sites
	rno-miR-375-3p	-0.02	Poorly conserved sites
	rno-miR-330-5p	-0.02	Poorly conserved sites
	rno-miR-326-3p	-0.02	Poorly conserved sites
	rno-miR-543-3p	-0.02	Poorly conserved sites
	rno-miR-551b-5p	-0.02	Poorly conserved sites
	rno-miR-449a-5p	-0.02	Poorly conserved sites
	rno-miR-488-5p	-0.02	Poorly conserved sites
	rno-miR-29b-5p	-0.02	Poorly conserved sites
	rno-miR-384-3p	-0.02	Poorly conserved sites
	rno-miR-742-3p	-0.02	Poorly conserved sites
	rno-miR-383-3p	-0.02	Poorly conserved sites
	rno-miR-493-5p	-0.02	Poorly conserved sites
	rno-miR-342-3p	-0.01	Poorly conserved sites
	rno-miR-126b	-0.01	Poorly conserved sites
	rno-miR-873-5p	-0.01	Poorly conserved sites
	rno-miR-493-5p	-0.01	Poorly conserved sites
	rno-miR-208a-5p	-0.01	Poorly conserved sites
	rno-miR-208b-5p	-0.01	Poorly conserved sites
	rno-miR-300-5p	-0.01	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

Hk2	rno-miR-153-3p	-0.01	Poorly conserved sites
	rno-miR-448-3p	-0.01	Poorly conserved sites
	rno-miR-873-5p	-0.01	Poorly conserved sites
	rno-miR-29b-5p	-0.01	Poorly conserved sites
	rno-miR-146b-5p	-0.01	Poorly conserved sites
	rno-miR-146a-5p	-0.01	Poorly conserved sites
	rno-miR-497-3p	-0.01	Poorly conserved sites
	rno-miR-297	-0.01	Poorly conserved sites
	rno-miR-1188-3p	-0.01	Poorly conserved sites
	rno-miR-770-5p	-0.01	Poorly conserved sites
	rno-miR-764-3p	-0.01	Poorly conserved sites
	rno-miR-452-3p	-0.01	Poorly conserved sites
	rno-miR-344b-3p	-0.01	Poorly conserved sites
	rno-miR-33-3p	-0.01	Poorly conserved sites
	rno-miR-320-5p	-0.01	Poorly conserved sites
	rno-miR-294	-0.01	Poorly conserved sites
	rno-miR-3065-3p	-0.01	Poorly conserved sites
	rno-miR-222-5p	-0.01	Poorly conserved sites
	rno-miR-3591	-0.01	Poorly conserved sites
	rno-miR-217-5p	-0.01	Poorly conserved sites
	rno-miR-30e-3p	-0.01	Poorly conserved sites
	rno-miR-30a-3p	-0.01	Poorly conserved sites
	rno-miR-30d-3p	-0.01	Poorly conserved sites
	rno-miR-141-5p	-0.01	Poorly conserved sites
	rno-miR-10a-3p	-0.01	Poorly conserved sites
	rno-miR-488-3p	-0.01	Poorly conserved sites
	rno-miR-3593-3p	-0.01	Poorly conserved sites
Hspb1	rno-miR-336-3p	-0.67	Poorly conserved sites
	rno-miR-509-3p	-0.4	Poorly conserved sites
	rno-miR-138-5p	-0.36	Poorly conserved sites
	rno-miR-138-1-3p	-0.34	Poorly conserved sites
	rno-miR-199a-3p	-0.32	Poorly conserved sites
	rno-miR-881-5p	-0.25	Poorly conserved sites
	rno-miR-871-5p	-0.24	Poorly conserved sites
	rno-miR-743a-5p	-0.24	Poorly conserved sites
	rno-miR-291a-5p	-0.21	Poorly conserved sites
	rno-miR-3559-5p	-0.17	Poorly conserved sites
	rno-miR-10b-3p	-0.17	Poorly conserved sites
	rno-miR-539-5p	-0.17	Poorly conserved sites
	rno-miR-667-5p	-0.81	Poorly conserved sites
	rno-miR-450a-5p	-0.77	Poorly conserved sites
	rno-miR-450b-5p	-0.77	Poorly conserved sites
	rno-miR-216b-3p	-0.5	Poorly conserved sites
	rno-miR-665	-0.46	Poorly conserved sites
	rno-miR-3594-3p	-0.44	Poorly conserved sites
	rno-miR-132-5p	-0.37	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

Nppa	rno-miR-551b-3p	-0.35	Poorly conserved sites
	rno-miR-362-3p	-0.29	Poorly conserved sites
	rno-miR-3572	-0.25	Poorly conserved sites
	rno-miR-488-3p	-0.25	Poorly conserved sites
	rno-miR-30b-3p	-0.24	Poorly conserved sites
	rno-miR-30c-1-3p	-0.24	Poorly conserved sites
	rno-miR-30c-2-3p	-0.24	Poorly conserved sites
	rno-miR-329-3p	-0.23	Poorly conserved sites
	rno-miR-194-3p	-0.2	Poorly conserved sites
	rno-miR-666-3p	-0.18	Poorly conserved sites
	rno-miR-759	-0.18	Poorly conserved sites
	rno-miR-463-3p	-0.17	Poorly conserved sites
	rno-miR-298-5p	-0.17	Poorly conserved sites
	rno-miR-3577	-0.17	Poorly conserved sites
	rno-miR-471-3p	-0.16	Poorly conserved sites
	rno-miR-542-3p	-0.15	Poorly conserved sites
Pla2g2a	rno-miR-92a-1-5p	-0.73	Poorly conserved sites
	rno-miR-877	-0.61	Poorly conserved sites
	rno-miR-296-3p	-0.59	Poorly conserved sites
	rno-miR-410-5p	-0.38	Poorly conserved sites
	rno-miR-653-3p	-0.36	Poorly conserved sites
	rno-miR-146b-3p	-0.35	Poorly conserved sites
	rno-miR-181b-2-3p	-0.34	Poorly conserved sites
	rno-miR-494-5p	-0.34	Poorly conserved sites
	rno-miR-3473	-0.33	Poorly conserved sites
	rno-miR-3075	-0.32	Poorly conserved sites
	rno-miR-6316	-0.32	Poorly conserved sites
	rno-miR-380-5p	-0.32	Poorly conserved sites
	rno-miR-496-5p	-0.32	Poorly conserved sites
	rno-miR-214-3p	-0.31	Poorly conserved sites
	rno-miR-711	-0.31	Poorly conserved sites
	rno-miR-3557-5p	-0.28	Poorly conserved sites
	rno-miR-336-3p	-0.27	Poorly conserved sites
	rno-miR-761	-0.25	Poorly conserved sites
	rno-miR-185-5p	-0.23	Poorly conserved sites
	rno-miR-219a-2-3p	-0.22	Poorly conserved sites
	rno-miR-107-5p	-0.21	Poorly conserved sites
	rno-miR-103-2-5p	-0.21	Poorly conserved sites
	rno-miR-103-1-5p	-0.15	Poorly conserved sites
	rno-miR-320-5p	-0.01	Poorly conserved sites
Txnip	rno-miR-666-5p	-0.52	Poorly conserved sites
	rno-miR-352	-0.51	Poorly conserved sites
	rno-miR-678	-0.48	Poorly conserved sites
	rno-miR-25-5p	-0.41	Poorly conserved sites
	rno-miR-26b-3p	-0.38	Poorly conserved sites
	rno-miR-6329	-0.38	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

Txnip	rno-miR-24-1-5p	-0.36	Poorly conserved sites
	rno-miR-24-2-5p	-0.36	Poorly conserved sites
	rno-miR-760-3p	-0.36	Poorly conserved sites
	rno-miR-3068-5p	-0.35	Poorly conserved sites
	rno-miR-675-5p	-0.33	Poorly conserved sites
	rno-miR-485-5p	-0.31	Poorly conserved sites
	rno-miR-3586-5p	-0.31	Poorly conserved sites
	rno-miR-339-5p	-0.31	Poorly conserved sites
	rno-miR-3552	-0.3	Poorly conserved sites
	rno-miR-154-3p	-0.29	Conserved sites
	rno-miR-6326	-0.29	Poorly conserved sites
	rno-miR-3075	-0.28	Poorly conserved sites
	rno-miR-493-3p	-0.27	Poorly conserved sites
	rno-miR-15a-3p	-0.27	Poorly conserved sites
	rno-miR-3587	-0.27	Poorly conserved sites
	rno-miR-141-3p	-0.27	Poorly conserved sites
	rno-miR-27a-5p	-0.27	Poorly conserved sites
	rno-miR-185-5p	-0.26	Poorly conserved sites
	rno-miR-6333	-0.26	Poorly conserved sites
	rno-miR-200a-3p	-0.26	Poorly conserved sites
	rno-miR-493-3p	-0.26	Poorly conserved sites
	rno-miR-3085	-0.26	Poorly conserved sites
	rno-miR-17-5p	-0.25	Conserved sites
	rno-miR-20b-5p	-0.25	Conserved sites
	rno-miR-3543	-0.25	Poorly conserved sites
	rno-miR-450b-3p	-0.25	Poorly conserved sites
	rno-miR-93-5p	-0.24	Conserved sites
	rno-miR-105	-0.24	Conserved sites
	rno-miR-106b-5p	-0.23	Conserved sites
	rno-miR-20a-5p	-0.23	Conserved sites
	rno-miR-291a-3p	-0.23	Conserved sites
	rno-miR-483-3p	-0.23	Poorly conserved sites
	rno-miR-1199-3p	-0.23	Poorly conserved sites
	rno-miR-3064-3p	-0.23	Poorly conserved sites
	rno-miR-20a-5p	-0.22	Conserved sites
	rno-miR-106b-5p	-0.22	Conserved sites
	rno-miR-204-3p	-0.22	Poorly conserved sites
	rno-miR-130a-5p	-0.22	Poorly conserved sites
	rno-miR-3541	-0.22	Poorly conserved sites
	rno-miR-1306-5p	-0.22	Poorly conserved sites
	rno-miR-93-5p	-0.21	Conserved sites
	rno-miR-17-5p	-0.21	Conserved sites
	rno-miR-20b-5p	-0.21	Conserved sites
	rno-miR-3558-3p	-0.21	Poorly conserved sites
	rno-miR-138-5p	-0.21	Poorly conserved sites
	rno-miR-1193-3p	-0.2	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

	rno-miR-325-5p	-0.2	Poorly conserved sites
	rno-miR-142-3p	-0.2	Poorly conserved sites
	rno-miR-129-5p	-0.2	Poorly conserved sites
Txnip	rno-miR-3566	-0.19	Poorly conserved sites
	rno-miR-183-5p	-0.19	Poorly conserved sites
	rno-miR-679	-0.19	Poorly conserved sites
	rno-miR-342-3p	-0.19	Poorly conserved sites
	rno-miR-3546	-0.18	Poorly conserved sites
	rno-miR-3064-3p	-0.17	Poorly conserved sites
	rno-miR-370-5p	-0.17	Poorly conserved sites
	rno-miR-147	-0.17	Poorly conserved sites
	rno-miR-352	-0.17	Poorly conserved sites
	rno-miR-3543	-0.17	Poorly conserved sites
	rno-miR-295-3p	-0.17	Poorly conserved sites
	rno-miR-667-3p	-0.17	Poorly conserved sites
	rno-miR-29a-5p	-0.16	Poorly conserved sites
	rno-miR-377-3p	-0.16	Poorly conserved sites
	rno-miR-337-3p	-0.16	Poorly conserved sites
	rno-miR-1199-5p	-0.15	Poorly conserved sites
	rno-miR-3556a	-0.15	Poorly conserved sites
	rno-miR-3556b	-0.15	Poorly conserved sites
	rno-miR-770-3p	-0.15	Poorly conserved sites
	rno-miR-6315	-0.15	Poorly conserved sites
	rno-miR-363-5p	-0.15	Poorly conserved sites
	rno-miR-342-5p	-0.15	Poorly conserved sites
	rno-miR-16-5p	-0.14	Conserved sites
	rno-miR-195-5p	-0.14	Conserved sites
	rno-miR-139-3p	-0.14	Poorly conserved sites
	rno-miR-652-5p	-0.14	Poorly conserved sites
	rno-miR-15b-5p	-0.13	Conserved sites
	rno-miR-494-3p	-0.13	Poorly conserved sites
	rno-miR-211-5p	-0.13	Poorly conserved sites
	rno-miR-204-5p	-0.13	Poorly conserved sites
	rno-miR-294	-0.13	Poorly conserved sites
	rno-miR-3552	-0.13	Poorly conserved sites
	rno-miR-322-5p	-0.12	Conserved sites
	rno-miR-879-5p	-0.12	Poorly conserved sites
	rno-miR-3068-5p	-0.12	Poorly conserved sites
	rno-miR-214-3p	-0.12	Poorly conserved sites
	rno-miR-193a-5p	-0.12	Poorly conserved sites
	rno-miR-122-5p	-0.12	Poorly conserved sites
	rno-miR-122-5p	-0.12	Poorly conserved sites
	rno-miR-327	-0.11	Poorly conserved sites
	rno-miR-17-2-3p	-0.11	Poorly conserved sites
	rno-miR-6314	-0.11	Poorly conserved sites
	rno-miR-1188-5p	-0.11	Poorly conserved sites

miRNA-mRNA analysis in diabetic cardiomyopathy

	rno-miR-1843a-5p	-0.11	Poorly conserved sites
	rno-miR-216a-5p	-0.11	Poorly conserved sites
	rno-miR-3557-3p	-0.11	Poorly conserved sites
	rno-miR-743a-3p	-0.11	Poorly conserved sites
	rno-miR-6216	-0.11	Poorly conserved sites
	rno-miR-96-3p	-0.11	Poorly conserved sites
	rno-miR-15a-5p	-0.1	Conserved sites
	rno-miR-141-3p	-0.1	Conserved sites
Txnip	rno-miR-22-3p	-0.1	Poorly conserved sites
	rno-miR-499-3p	-0.1	Poorly conserved sites
	rno-miR-204-3p	-0.1	Poorly conserved sites
	rno-miR-7a-1-3p	-0.1	Poorly conserved sites
	rno-miR-200a-3p	-0.09	Conserved sites
	rno-miR-22-5p	-0.09	Poorly conserved sites
	rno-miR-133a-5p	-0.09	Poorly conserved sites
	rno-miR-320-3p	-0.09	Poorly conserved sites
	rno-miR-376b-3p	-0.09	Poorly conserved sites
	rno-miR-3068-5p	-0.09	Poorly conserved sites
	rno-miR-3065-5p	-0.09	Poorly conserved sites
	rno-miR-497-5p	-0.08	Conserved sites
	rno-miR-3084a-5p	-0.08	Poorly conserved sites
	rno-miR-542-3p	-0.08	Poorly conserved sites
	rno-miR-3557-3p	-0.08	Poorly conserved sites
	rno-miR-3575	-0.08	Poorly conserved sites
	rno-miR-653-3p	-0.08	Poorly conserved sites
	rno-miR-181b-2-3p	-0.08	Poorly conserved sites
	rno-miR-20b-3p	-0.08	Poorly conserved sites
	rno-miR-17-1-3p	-0.08	Poorly conserved sites
	rno-miR-207	-0.08	Poorly conserved sites
	rno-miR-152-3p	-0.07	Conserved sites
	rno-miR-148b-3p	-0.07	Conserved sites
	rno-miR-1188-3p	-0.07	Poorly conserved sites
	rno-miR-130b-5p	-0.07	Poorly conserved sites
	rno-miR-141-5p	-0.07	Poorly conserved sites
	rno-miR-186-3p	-0.07	Poorly conserved sites
	rno-miR-761	-0.07	Poorly conserved sites
	rno-miR-216b-5p	-0.07	Poorly conserved sites
	rno-miR-466d	-0.07	Poorly conserved sites
	rno-miR-295-3p	-0.07	Poorly conserved sites
	rno-miR-148a-3p	-0.06	Conserved sites
	rno-miR-191a-3p	-0.06	Poorly conserved sites
	rno-miR-6326	-0.06	Poorly conserved sites
	rno-miR-3552	-0.06	Poorly conserved sites
	rno-miR-150-5p	-0.06	Poorly conserved sites
	rno-miR-150-5p	-0.06	Poorly conserved sites
	rno-miR-205	-0.06	Poorly conserved sites

	rno-miR-149-5p	-0.06	Poorly conserved sites
	rno-miR-3568	-0.05	Poorly conserved sites
	rno-miR-532-3p	-0.05	Poorly conserved sites
	rno-miR-3564	-0.04	Poorly conserved sites
	rno-miR-3085	-0.04	Poorly conserved sites
	rno-miR-3573-3p	-0.04	Poorly conserved sites
	rno-miR-301a-5p	-0.03	Poorly conserved sites
	rno-miR-204-3p	-0.03	Poorly conserved sites
	rno-miR-301a-5p	-0.03	Poorly conserved sites
	rno-miR-301b-5p	-0.03	Poorly conserved sites
	rno-miR-653-5p	-0.02	Poorly conserved sites
	rno-miR-301b-5p	-0.02	Poorly conserved sites
	rno-miR-873-3p	-0.02	Poorly conserved sites
Txnip	rno-miR-3074	-0.02	Poorly conserved sites
	rno-miR-181b-5p	-0.02	Poorly conserved sites
	rno-miR-181d-5p	-0.02	Poorly conserved sites
	rno-miR-181a-5p	-0.02	Poorly conserved sites
	rno-miR-181c-5p	-0.02	Poorly conserved sites
	rno-miR-19b-1-5p	-0.02	Poorly conserved sites
	rno-miR-19b-2-5p	-0.02	Poorly conserved sites
	rno-miR-33-3p	-0.02	Poorly conserved sites
	rno-miR-9a-3p	-0.02	Poorly conserved sites
	rno-miR-214-3p	-0.02	Poorly conserved sites
	rno-miR-449c-3p	-0.02	Poorly conserved sites
	rno-miR-153-5p	-0.01	Poorly conserved sites
	rno-miR-294	-0.01	Poorly conserved sites
	rno-miR-673-5p	-0.01	Poorly conserved sites
	rno-miR-27a-3p	-0.01	Poorly conserved sites
	rno-miR-27b-3p	-0.01	Poorly conserved sites
	rno-miR-532-3p	-0.01	Poorly conserved sites
	rno-miR-668	-0.01	Poorly conserved sites
	rno-miR-3561-5p	-0.01	Poorly conserved sites
	rno-miR-761	-0.01	Poorly conserved sites

and could be regulated by rno-mir-214 (LogFC -1.2.5), rno-mir-320 (LogFC -1.1.8), rno-mir-877 (LogFC -1.0.1), considering their regulations and TargetScan 7.1. web dataset data (Table 2 and Table 4).

The mRNA-mRNA and miRNA-mRNA interactions according to the IPA software and TargetScan 7.1. web dataset are shown at Figure 3. Possible pathways associated with DCM development: gene-gene: *Agtr1-Nppa*, *Agtr1-Txnip*; miRNA-target mRNAs: *Agtr1* (rno-miR-7a, rno-miR-194, rno-mir-188, rno-mir-204 and rno-mir-466c); *Fstl1* (rno-mir-203, rno-mir-322, rno-mir-22, rno-mir-200c, rno-mir-342, rno-mir-877, rno-mir-204, rno-mir-92b, rno-mir-214, rno-miR-30-c2*, rno-miR-7a, rno-miR-17-3p, rno-mir-

20b, rno-mir-186 and rno-mir-320); *Txnip* (rno-mir-20b, rno-mir-204, rno-mir-342, rno-mir-322, rno-mir-214, rno-miR-7a, rno-mir-22, rno-mir-320, rno-mir-186, rno-mir-148b, rno-mir-532 and rno-miR-17-3p); *Pla2g2a* (rno-mir-214, rno-mir-320 and rno-mir-877); *Hk2* (rno-mir-34a, rno-mir-532, rno-mir-21, rno-miR-17-3p, rno-mir-322, rno-mir-187, rno-mir-188, rno-mir-214, rno-mir-320, rno-mir-184 and rno-mir-342); *Hspb1* (rno-mir-199a); *Nppa* (rno-miR-30-c2* and rno-miR-194) and miRNA-miRNA: rno-mir-214-rno-mir-148.

5. DISCUSSION

In this study, *Pla2g2a* was up-regulated in LV of diabetic rats in an acute and chronic condition, which

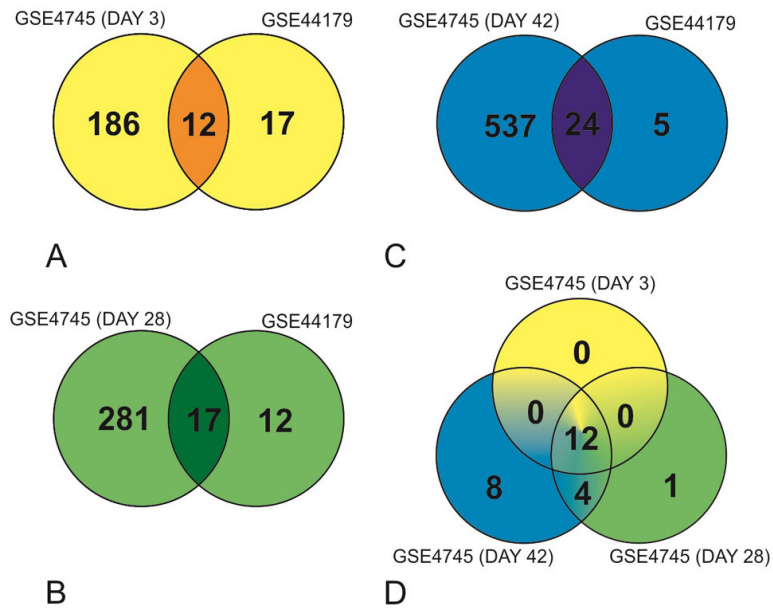


Figure 2. Venn diagram of MICRORNA-detected regulatory miRNAs of DCM-related mRNAs differentially expressed in LV of STZ-induced diabetic rats (GSE4745) and miRNAs differentially expressed in LV of STZ-induced and high-fat diet diabetic rats (GSE44179). A, GSE4745 (day 3) vs. GSE44179; B, GSE4745 (day 28) vs GSE44179; C, GSE4745 (day 42) vs GSE44179; D, Intersection of all periods evaluated in GSE4745.

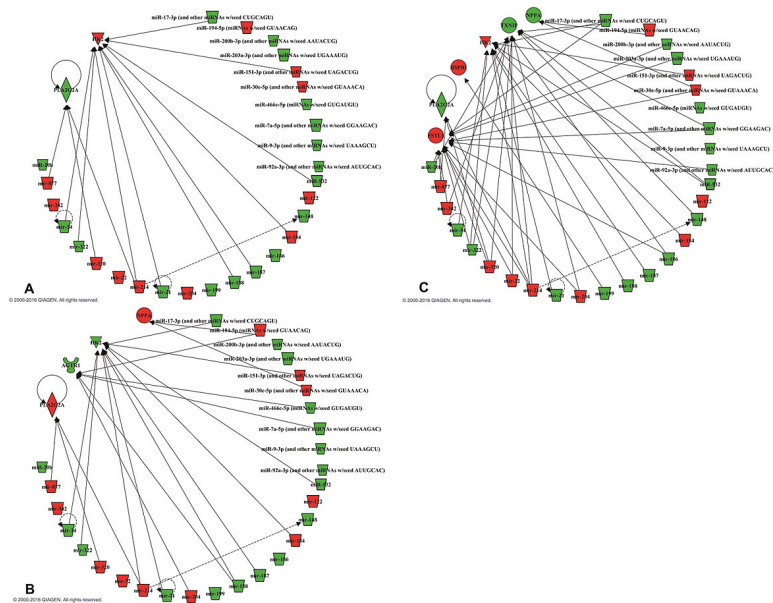


Figure 3. IPA and MICRORNA network of miRNAs-mRNAs interactions associated with diabetes-induced cardiotoxicity in periods evaluated in GSE4745. A - Day 3 after STZ-induction; B - Day 28 after STZ-induction and C - Day 42 after STZ-induction.

is suggestive that it may be involved in LV dysfunction induced by hyperglycemia. PLA2G2A is a phospholipase A2 group II A which catalyzes the hydrolyzes of the acyl group at the sn-2 position of glycerophospholipids forming non-esterified fatty acids and lysophospholipids and plays a critical role in inflammation (25). Transgenic mice expressing an extracellular group IIa phospholipase A2 (sPLA2) have a higher number of atherosclerotic lesions when maintained on a high-fat, high-cholesterol and a low-fat chow diet in comparison

with non-transgenic littermates. Immunohistochemical staining indicated that sPLA2 was present in the atherosclerotic lesions of the transgenic mice, which suggests that sPLA2 may promote atherogenesis, in part, through its effects on lipoprotein levels, increasing LDL cholesterol and decreasing HDL cholesterol. These data are suggestive that increased sPLA2 is involved in lipid-driven atherosclerosis, which is an important risk factor for CAD and possible other chronic inflammatory diseases (26).

Another study involving the measurement of serum levels of sPLA2 activity and secretory phospholipase A₂ type IIA (sPLA2-IIA) evaluated the increase of circulating sPLA2-activity and sPLA2-IIA in patients with stable CAD suggesting their relation with functional characteristics of coronary stenosis in these patients. Measuring these molecules could be a possibility to predict the severity of CAD (27). The prognostic value of the sPLA2 activity in patients with an acute coronary syndrome (ACS) was determined through a measurement of sPLA2 antigen levels and its activity in plasma samples. The results showed that the high plasma sPLA2 activity is a major independent predictor of death and new or recurrent myocardial infarction (MI) in patients with ACS (28). Based on these previous studies and the hyperglycemia-induced *Pla2g2a* up-regulation in rats LV, it is likely that this gene plays an important role in the pathophysiology of DCM.

Pla2g2a regulatory miRNAs, rno-miR-877, rno-miR-320 and rno-miR-214, were down-regulated in LV of rats exposed to a high glucose concentration, suggesting their involvement in the development of DCM. Despite the down regulation of rno-miR-877 in ventricles of diabetic rats observed in GSE44179 dataset, there are only a few studies about these miRNA, especially in DCM. Researchers evaluated the expression profiles of miRNAs in single-cell suspensions in the lung prepared from C57BL/6 mice, before and after myofibroblast differentiation of lung resident mesenchymal stem cells and observed that the miR-877-3p is highly up-regulated in the myofibroblast differentiation and in the fibrotic lung. In addition, they found that miR-877-3p sequestration inhibited the myofibroblast differentiation, suggesting a potential application of miR-877-3p as a fibrosis suppressor in pulmonary fibrosis therapy and as a fibrosis marker for predicting prognosis, which indentified that miR-877-3p, is a miRNA that acts as a supressor in pulmonary fibrosis (29).

Regarding rno-miR-320, an *in vivo* study using an animal model evaluated the role of rno-miR-320 in ischemia/reperfusion (I/R). They observed that miR-320 expression worsens myocardial I/R injury, while its inhibition protects against myocardial apoptosis, mainly via the Insulin Growth Factor 1 (IGF-1) pathway, affecting the levels of antiapoptotic signaling pathways. This suggests miR-320 may represent a valuable tool and potential therapeutic target for protection against I/R induced cardiac injury (30). Although, in our integrative analysis, the rno-miR-320 was down-regulated in DCM, the association of this miRNA with apoptosis may represent an important finding.

Rno-miR-214 is a bi-functional cardio-miR, which is involved in cardiac physiology (31). In the

dataset analyzed, rno-miR-214 was down-regulated in LV of diabetic rats, which is suggestive of its role in DCM through the regulation of the *Pla2g2a* gene expression. Supporting our hypothesis, the miR-214 was also observed down-regulated in blood samples of patients with acute MI, and angina pectoris, suggesting that the low expression of hsa-miR-214 is associated with severity of coronary stenosis and it could be a promising biomarker for CAD (32). Moreover, a model of mmu-miR-214 knockout mice showed a loss of cardiac contractility, increased apoptosis, and excessive fibrosis in response to ischemia/reperfusion injury (33), supporting the role of this miR in DCM complication.

The analysis also showed that *Hk2* mRNA was down-regulated and related to the high expression of its regulatory miRNAs (rno-miR-17, rno-miR-187, rno-miR-34a, rno-miR-322, rno-miR-188, rno-miR-532 and rno-miR-21) in LV of rats exposed to a high glucose concentration, suggesting their important role in the pathophysiology of DCM.

Hk2 is an isozyme which occurs in mammalian tissue and catalyzes glucose phosphorylation. It has an affinity for glucose and product inhibition for glucose-6-phosphate (34,35). Additionally, a study observed the role of mitochondria-bound hexokinase 2 (mTHK2) in cardio protection using hearts of ischemic preconditioning rats whose results suggest that mTHK2 stimulates reactive oxygen species (ROS) production and mitochondrial permeability transition pore opening on reperfusion, leading to MI, during ischemia (36). Moreover, a study using an animal model of heterozygous HK2-deficient mice displayed increased hypertrophy and heart failure in response to transverse aortic constriction, as well as, there being an increased ROS production and *de novo* hypertrophy, suggesting that the use of some methods to increase the HK2 levels could control the cardiac hypertrophy (37).

As for the regulatory miRNAs of Hk2, an *in vitro* study was performed to detect if whether the miR-17-5p is differentially expressed in ischemia and reperfusion mice model and neonatal rat ventricular cardiomyocytes (NRCVs) under oxidative stress. The investigators suggested that overexpression of miR-17-5p increases the damage in cardiomyocyte through reduction of cell viability and increasing apoptosis (38), this result reinforces the correlation of rno-miR-17 with apoptosis in cardiomyocytes and it relation with *Hk2* mRNA. The hsa-miR-187 was found differentially expressed in studies that analyzed doxorubicin (DOX)-cardiotoxicity induced in cell culture. They showed an early deregulation in miR-187 expression by real-time PCR analyses in human-induced pluripotent stem cell-derived cardiomyocytes exposed to DOX which was very important and links this miRNA to its use as an early sensitive cardiotoxicity biomarker (39, 40).

Regarding rno-miR-34, its miRNA has been associated with cardiac problems (41,42). In a previous *in vivo* study that induced cardiac injury in neonatal and adult hearts, it was observed that miR-34 could act in cardiac repair and regeneration through its regulation of mRNA targets such as *B-cell lymphoma 2 (Bcl2)*, *Cyclin D1* and *Sirtuin 1 (Sirt1)*, and are related with the cell apoptosis process (41). In addition, in an *in vitro* study using an *in vitro* anoxia and reoxygenation injury (ARI) model based on rat heart-derived H9c2 cells, it has suggested an important relation of it miRNA in cardiac function. The cells were treated with Resveratrol, a natural polyphenolic compound, which suppresses the effects of rno-miR-34a upregulation in anoxia and reoxygenation injury, suggesting a cardiac protective effect on cardiomyocytes in ARI (42).

Study using RNA sequencing also corroborates with our *in silico* results, showing an upregulation of rno-miR-322-3p in *in vitro* study using neonatal heart cells in hypertrophy model (43). In cardiac injury, the expression of rno-miR-322 *in vivo* was measured in the rat carotid artery after injury and *in vitro* in rat vascular smooth muscle cells (VSMC) proliferation and in both analyses, the rno-miR-322 were up-regulated after vascular injury, suggesting that the miRNA could be a useful therapeutic target to inhibit VSMC dedifferentiation during a vascular occlusive disease (44).

Regarding rno-miR-21-5p, an unbiased quantitative miRNA microarray analysis the right and left ventricles of normal and pulmonary arterial hypertension rats observed an upregulation of rno-miR-21-5p in a pulmonary arterial hypertension condition (45). In mice, the mmu-miR-21-5p has been associated with increased cardiac fibrosis, and its upregulation was observed in the heart of a myocardial infarction animal model, suggesting that this miRNA may act regulating the suppression in TGF- β via, and consequently increasing the production of collagen (46).

Rno-miR-188-3p participates in the suppression of autophagy and myocardial infarction by targeting ATG7 in an *in vitro* and *in vivo* model of myocardial infarction in rats (47). Another study of miRNA microarray assay concludes that rno-miR-188 is the most downregulated miRNA in homocysteine cardiac remodeling *in vitro* model which suggests that it miRNA acts in cardiac remodeling in cardiovascular diseases (48).

The association between miR-532 and heart disease was observed in a study evaluating miRNAs levels in endothelial-like cells of human volunteers. The endothelial-like cells were cultured under hypoxia and normoxia conditions and the hsa-miR-532 was up-regulated under hypoxia conditions when compared to normoxia conditions (49).

Therefore, up regulation of rno-miR-17, rno-miR-187, rno-miR-34a, rno-miR-322, rno-miR-188, rno-miR-532 and rno-miR-21 and down regulation of the *Hk2* mRNA target could lead to a loss of cardio protection and consequently to the development of cardiac complications, including those associated with a hyperglycemic condition.

Despite the interesting findings of this *in silico* study, there are some limitations including the need to validate data from hyperglycemia-induced mRNAs and regulatory miRNAs interactions and roles in experimental models. Besides our focusing on hyperglycemia effects, two different animal models of diabetes (type 1 and 2) were used to investigate mRNA and miRNAs differentially expressed in LV rats. However, it has been shown that both models develop hyperglycemia leading to structural and functional alterations in LV in both types of diabetes (50).

In conclusion, the differential expression of *Pla2g2a* and *Hk2* mRNA and their interactions with regulatory miRNAs rno-miR-877, rno-miR-320 and rno-miR-214 (*Pla2g2a*) and rno-miR-17, rno-miR-187, rno-miR-34a, rno-miR-322, rno-miR-188, rno-miR-532 and rno-miR-21 (*Hk2*) could be associated with DCM in rats. These miRNAs and mRNAs targets may be useful biomarkers to detect early cardiovascular complications of diabetic patients.

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