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Original Article

Evaluation of miRNAs regulation of BDNF and IGF1 genes in T2DM insulin resistance in experimental models: bioinformatics based approach

Avaliação da regulação por miRNAs dos genes BDNF e IGF1 na resistência a insulina no diabetes do tipo 2 em modelos experimentais: *in silico*

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Abstract

microRNAs (miRNAs) are recognized as diabetes mellitus type 2 (T2DM) biomarkers useful for disease metabolism comprehension and have great potential as therapeutics targets. BDNF and IGF1 increased expression are highly involved in the benefits of insulin and glucose paths, however, they are down-regulated in insulin resistance conditions, while their expression increase is correlated to the improvement of glucose and insulin metabolism. Studies suggest the microRNA regulation of these genes in several different contexts, providing a novel investigation approach for comprehending T2DM metabolism and revealing potential therapeutic targets. In the present study, we investigate in different animal models (human, rat, and mouse) miRNAs that target BDNF and IGF1 in skeletal muscle tissue with T2DM physiological conditions. Bioinformatics tools and databases were used to miRNA prediction, molecular homology, experimental validation of interactions, expression in the studied physiological condition, and network interaction. The findings showed three miRNAs candidates for IGF1(miR-29a, miR-29b, and miR-29c) and one for BDNF (miR-206). The experimental evaluations and the search for the expression in skeletal muscle from T2DM subjects confirmed the predicted interaction between miRNA-mRNA for miR-29b and miR-206 through human, rat, and mouse models. This interaction was reaffirmed in multiple network analyses. In conclusion, our results show the regulation relationship between miR-29b and miR-206 with the investigated genes, in several tissues, suggesting an inhibition pattern. Nevertheless, these data show a large number of possible interaction physiological processes, for future biotechnological prospects.

Keywords: miRNAs prediction, T2DM, IGF1/BDNF, therapeutics, interaction validation.

Resumo

Os microRNAs (miRNAs) são reconhecidos como biomarcadores do diabetes mellitus tipo 2 (DM2), úteis para a compreensão do metabolismo da doença, e possuem grande potencial como alvos terapêuticos. O aumento da expressão de BDNF e IGF1 está altamente envolvido nos benefícios as vias de insulina e glicose, porém, são regulados negativamente em condições de resistência à insulina, enquanto seu aumento de expressão está correlacionado com a melhora do metabolismo da glicose e da insulina. Estudos sugerem a regulação desses genes por microRNA em vários contextos diferentes, proporcionando uma nova abordagem de investigação para compreender o metabolismo do DM2 e revelar potenciais alvos terapêuticos. No presente estudo, investigamos em diferentes modelos animais (humanos, ratos e camundongos) miRNAs que têm como alvo BDNF e IGF1 em tecido muscular esquelético com condições fisiológicas de DM2. As análises foram realizadas utilizando ferramentas de bioinformática e bancos de dados para predição de miRNA, homologia molecular, validação experimental de interações, expressão na condição fisiológica estudada e interação em rede. Os resultados mostraram três candidatos a miRNAs para IGF1 (miR-29a, miR-29b e miR-29c) e um para BDNF (miR-206). As avaliações experimentais e a busca pela expressão no músculo esquelético de indivíduos com DM2 confirmaram a interação prevista entre miRNA-mRNA para miR-29b e miR-206 através de modelos humanos, ratos e camundongos. Essa interação foi reafirmada em múltiplas

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análises de rede. Em conclusão, nossos resultados mostram a relação de regulação entre miR-29b e miR-206 com os genes investigados, em diversos tecidos, sugerindo um padrão de inibição. Contudo, esses dados mostram um grande número de possíveis processos fisiológicos de interação para perspectivas biotecnológicas.

Palavras-chave: predição de miRNAS, DM2, IGF1/BDNF, terapêutica, validação de interação.

1. Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules evolutionarily conserved, with approximately 22 nucleotides. They are involved in regulating numerous biological processes through post-transcription silencing mechanisms. These molecules can recognize and target specific messenger RNA (mRNA) by base pairing in seed sequence regions, a conserved heptameric sequence mainly situated at positions 2-7 from the miRNA 5´-end. These molecules lead to an inhibition mechanism mediated by a protein complex with endonucleases that cleavage the target causing its degradation and consequent translation repression (O'Brien et al., 2018).

The miRNAs are well known for their regulation roles in illnesses such as cancer, acting as tumor suppressors; in heart disorders, working like critical regulators of cardiovascular function, and neurodegenerative diseases, through the regulation of gene expression, being described as biomarkers for prognostics and diagnostics (Vishnoi and Rani, 2017). The knowledge about the mechanisms trigged by miRNAs is being elucidated for therapeutic uses, usually through the expression modulation causing its inhibition using designed specific small interference RNAs (siRNAs) or "antagomirs" (Krützfeldt et al., 2005).

Type 2 Diabetes Mellitus (T2DM) is a worldwide disorder strictly related to the subject lifestyle, being recurrent in overweight and sedentary individuals. T2DM disorder is characterized by hyperglycemic clinical conditions mediated by insulin functioning failures in target tissues (Kharroubi and Darwish, 2015). One of the main issues is insulin resistance (IR), characterized by the decrease of the cell sensibility to insulin action response, disabling the glucose capture by tissues, which leads to the increase of glucose in the blood flow. IR is highly related to T2DM and metabolic syndrome (Sinaiko and Caprio, 2012).

The genes BDNF and IGF1 have been related to improvements in glucose homeostasis and have an impressive expression regulation decrease in IR conditions (McDonald et al., 2007; Rozanska et al., 2020). In the T2DM profile, these genes are down-regulated, enabling their action through signaling beneficial mechanisms in the disorder pathogenesis (Fujinami et al., 2008; Suda et al., 2016). BDNF and IGF1 are genes that present a history of regulation by miRNAs in several different tissues (Numakawa et al., 2011; Jung and Suh, 2015).

miRNAs have an altered profile in T2DM, being useful as biomarkers for prognosis and diagnostics. Nevertheless, studies indicate that this non-coding RNA class is important for understanding the mechanisms involved in T2DM pathogenesis (Kim and Zhang, 2019). So, the investigation of specific miRNAs that target key genes can provide novel data for the comprehension of miRNA's role in T2DM and, through its interpretation, can reveal the metabolic paths and networks that they are involved which can allow the selection of potential candidates for future therapeutic approaches elaboration.

Thus, in the present, we explore the novelty of bioinformatics and data mining tools in view to provide an accurate and relevant investigation of the regulation by miRNAs in T2DM metabolism through the evaluation of these molecules in BDNF and IGF1 targeting, and the networks paths evolved. The study also prospects future *in vivo* application, so, for this, we performed an aggregated organisms approach for *Homo sapiens*, *Rattus norvegicus* and *Mus musculus*.

2. Methodology

2.1. Prediction of miRNAs candidates

The miRNAs prediction evaluation was performed in an aggregated methodology approach using the web-based bioinformatics sources: TargetScan (2021), miRDB (2021), and microT-CDS (Diana Lab, 2021) (Agarwal et al., 2015; Chen and Wang, 2020; Paraskevopoulou et al., 2013).

The miRNAs were selected according to the following statistical measures: TargerScan - score percentile ≳ 90%; miRDB - target score ≳ 90 and microT-CDS - miTG score ≳ 0.90. These variables are based on the probability of miRNAs repressing the target gene. The score percentile calculates the most favorable miRNA repression sites, the target score represents the confidence level of target prediction. In contrast, the miTG score is a general score for the predicted interaction, where the closer to 1, the better the confidence.

In view to produce a multiple organism approach, the query used for this predictions were the homologous genes: BDNF (ENSG00000176697) and IGF1 (ENSG00000017427) from humans (*Homo sapiens*); *Igf1* (ENSRNOG00000004517) and *Bdnf* (ENSRNOG00000047466) from rat (*Rattus norvegicus*), and *Igf1* (ENSMUSG00000020053) and *Bdnf* – (ENSMUSG00000048482) from mouse (*Mus musculus*).

The results of the prediction were separated by organism and tool in a Venn diagram using the InteractiVenn (2021) web-based tool (Heberle et al., 2015). The results were compared to select the miRNAs that were recurrent between the different prediction tools for the same organism, and later these results were confronted in view to select miRNAs recurrent between the three organisms.

2.2. miRNA conservation evaluation through the studied organisms

The miRNAs selected were compared for their similarity in nucleotide size, sequence, and chromosomic location through human, rat, and mouse genomes. The seed binding in the three target organisms was also compared using

the TargetScan algorithm to select the most statistically significant binding site for each miRNA-mRNA combination (Agarwal et al., 2015).

2.3. miRNA-mRNA interaction validation

Were used the databases miRTArbase (2021) e TarBase V.08 (DIANA Tools, 2021) to investigate the experimental validation of the interactions between the selected miRNAs and the target genes. This evaluation were considerate the three organisms, target tissues, and the methodology used to confirm the interaction (Huang et al., 2020; Karagkouni et al., 2018).

2.4. Differential expression in T2DM skeletal muscle

Genome Expression Omnibus (GEO) (NCBI, 2021) were used to confront miRNAs candidates with datasets about healthy and T2DM conditions from skeletal muscle tissue of humans, rat, and mouse (Edgar et al., 2002). The skeletal muscle tissue was chosen due to its importance in the T2DM context, being one of the most affected organs in insulin resistance conditions and is highly deteriorated since it is responsible for the majority of insulin-stimulated body glucose disposal (Phielix and Mensink, 2008). This evaluation was considered *in vitro* and *in vivo* studies, where were identified the expression profile of the selected miRNAs in the T2DM condition.

2.5. Network multiple analyses

In miRnet (2021) platform were performed multiple evaluations for interactions between target genes, miRNAs selected, and other molecules in the genome, in view to identify the network relationship paths evolved. From that were investigated gene ontology (GO: BP) and diseases that were statistically related to the cluster by the same tool (Chang et al., 2020).

All the steps mentioned in the methodology are described in Figure 1.

3. Results

3.1. In silico selection of the miRNAs candidates for targeting IGF1 and BDNF genes in humans, mouse, and rats

3.1.1. IGF-1 – miRNA target predictive evaluation

The predictive analyses of IGF1 (Figure 2) showed a total of 423 miRNAs, among these 260 were for humans (Figure 2A), 123 for a mouse (Figure 2B), and 40 for rat (Figure 2C). The comparison between the results of the different organisms' (Figure 2. D and Table 1) showed miR-29a, miR-29b, and miR-29c were recurrent for the three animal models. The list of the predicted miRNAs for IGF1 from each tool and organism is available in Table S1 of the Supplementary material.

The comparations between the different tools and organisms (Figure 2, Table 1), showed in human (Figure 2A) 15 equal miRNAs between the tools, while in mouse and rat, the prediction evaluation reported only three equal miRNAs, each one. Between all organisms studied, three miRNAs were equal (miR-29a, miR-29b, and miR-29c), all from the same family.

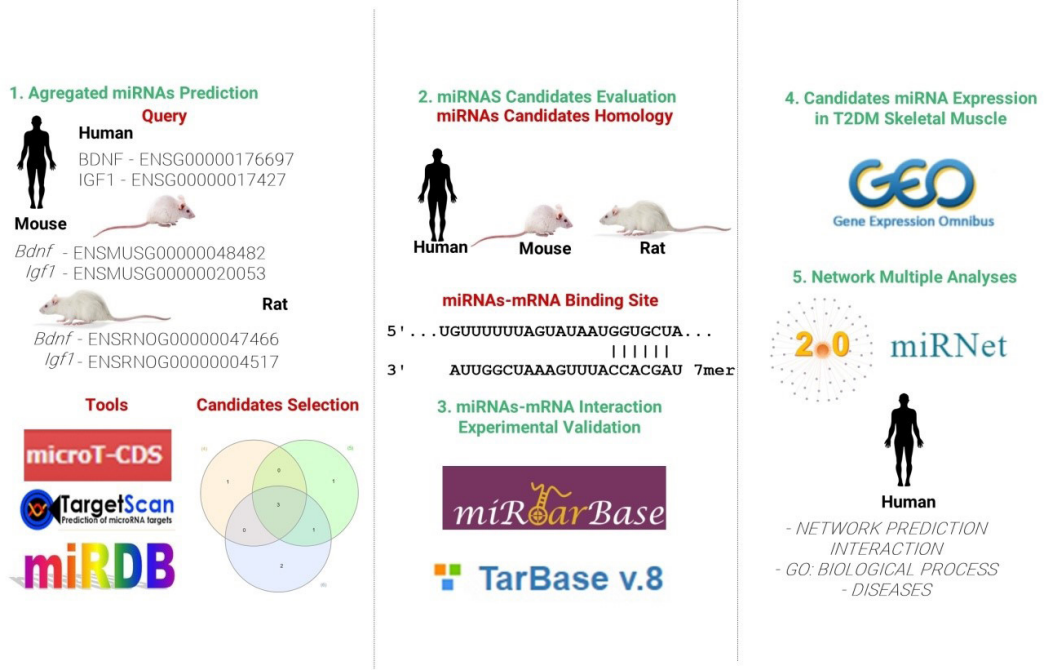

Figure 1. Methodological pipeline followed in the study.

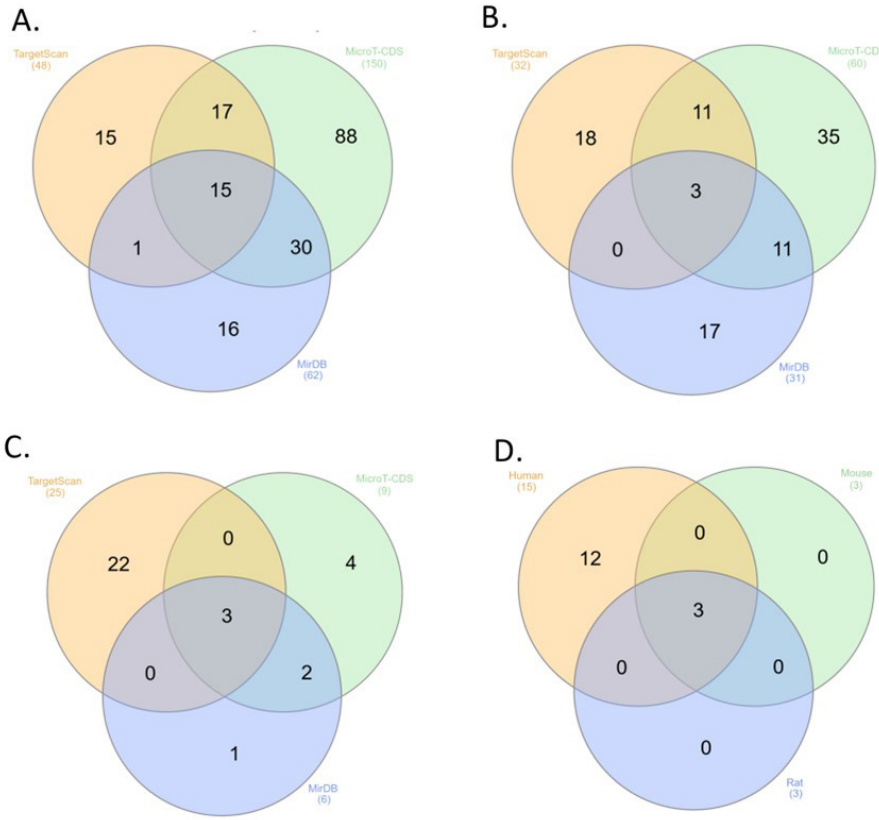

Figure 2. Comparative Prediction for miRNA that targets IGF1 genes. (A) IGF1 – Human; (B) Igf1 – Mouse; (C) Igf1 – Rat; (D) Comparative results from Human, mouse, and rat.

Table 1. List of miRNAs obtained in the comparative prediction analyses using IGF1 as target for human, mouse and rat.

Human (hsa)	***Mouse (mmu)***	***Rat (rno)***	***All***
miR-206, miR-603, miR-1-3p	miR-1b, miR-206, miR-495	miR-10a, miR-1, miR-1b, miR-206, miR-495	miR-206

3.1.2. BDNF – miRNA target predictive evaluation

BDNF evaluation (Figure 3) presented a total of 259 miRNAs, 102 of them, were from human (Figure 3A), 120 mouse (Figure 3B), and 37 rat (Figure 3C). Moreover, the evaluation comparing these results (Figure 3D, Table 2), showed mir-206 as the only predicted miRNA recurrent to target BDNF in the three organisms. The list of the predicted miRNAs for BDNF from each tool and organism is available in Table S2.

In human and mouse evaluation, three miRNAs were found recurrent between the results of the tools. In comparison, mouse presented a total of three and rat four. These evaluation results are disposable in Table 2. In the present evaluation, were not found match results in miRNA target prediction for *Bdnf* gene in rat using microT-CDS.

3.2. Molecular homology between the miRNAs candidates in the query organisms

The evaluation of molecular conservation investigated the similarity between the predicted miRNAs candidates through human, mouse, and rat genomes (Table 3). The results demonstrated an exact similarity for miR-206 and the three miR-29 family members in sequence size and nucleotide number in the organisms studied. However, all the chromosomic locations were different.

3.3. miRNA-mRNA seed binding region through the human, mouse, and rat genomes

The evaluation of the miRNA-mRNA interaction position is described in Tables 4 and 5. The findings demonstrated a common sequence site of recurrent seven nucleotides (UGGUGCU) in the 8mer seed region, between the miRNAs (miR-29a, miR-29b, and miR-29c) and the IGF1 genes from the animal models.

In miR-206 evaluation (Table 5), the interaction with BDNF genes among the studied species demonstrated that the binding site was at the 7mer-m8 seed region, where the seed sequence was equal for human, rat, and mouse (UGUAAGG). However, the miRNA-mRNA binding position in each organism was different for the four miRNAs candidates to target the studied genes.

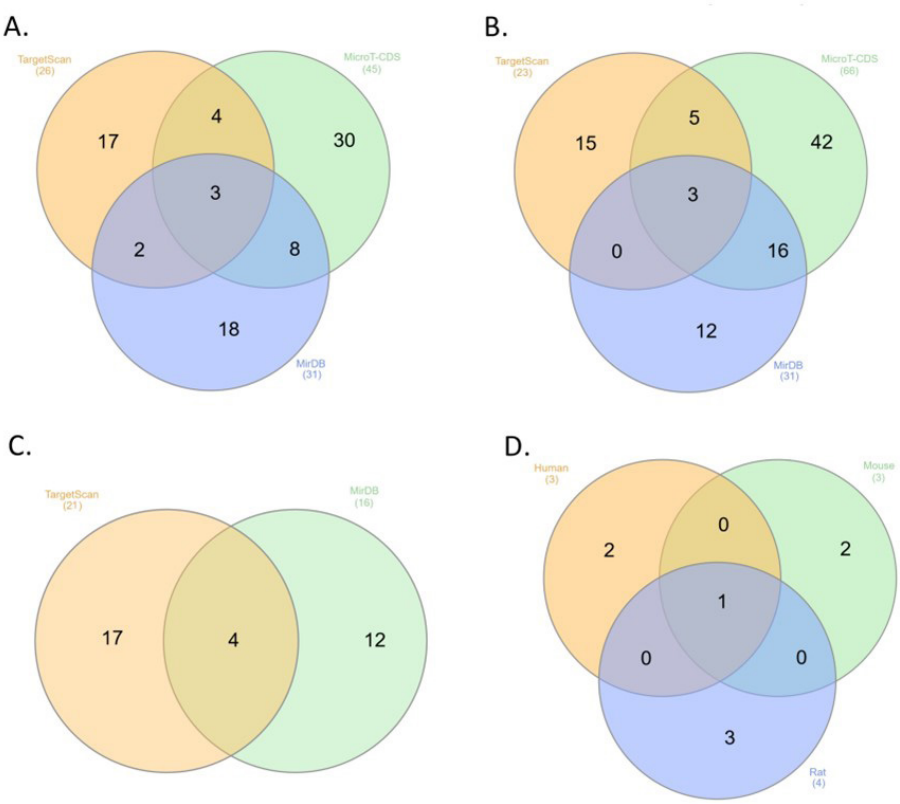

Figure 3. Comparative Prediction for miRNA that targets BDNF genes. (A) Human; (B) Mouse; (C) Rat; (D) Comparative results from Human, mouse, and rat).

Table 2. List of miRNAs obtained in the comparative prediction analyses using IGF1 as target for human, mouse and rat.

Human (hsa)	***Mouse (mmu)***	***Rat (rno)***	***All***
miR-301b, miR-301a, miR-130b, miR-130a, miR-4295, miR-3666, miR-454, miR-19a, miR-19b, miR-29b, miR-29a, miR-29c, miR-495, miR-5590, miR-486.	miR-1a, miR-206, miR-325, miR-29c, miR-29b, miR-29a, let-7e-5p	miR-29c, miR-29b, miR-29a	miR-29a, miR-29b, miR-29c

3.3.1. IGF1 genes interaction with miR-29a, miR-29b, and miR-29c in human, rat, and mouse

All the three miRNAs selected presented interactions with IGF1 genes by experimental approaches (Tables 6 and 7), but only in humans and mouse, in rats, the interaction was not described by any of the investigated databases.

Reports from humans showed miR-29a and IGF1 gene interaction in the epididymis and the brain. The confirmation methodology for the first tissue was Luciferase reporter assay, qRT-PCR and Western blot. In the brain, the validation was performed with HITS-CLIP technology. miR-29b and miR-29c were also reported to target IGF1 in the brain by the same method.

Data from mouse experiments demonstrated that miR-29a targets *Igf1* in the brain and embryo tissue. Both results were findings achieved by the use of the luciferase reporter assay method. Meanwhile, miR-29b was shown to target *Igf1* in the brain, muscle (C2C12 cells), and liver. In the first, interactions were confirmed by ELISA, luciferase reporter assay, and qRT-PCR. In the others, interactions were validated by HITS-CLIP technology.

3.3.2. BDNF genes interaction with miR-206 in human, rat, and mouse

The search for the interactions from miR-206 for target BDNF genes miR-206 in experimentally validated experiments (Table 8), resulted in the confirmation of this interaction in human, mouse, and rat by miRTarbase database. However, in TarBase V.08. was not found the same results.

Table 3. miR-29 family and miR-206 conserved through human, mouse and rat.

Organism	*Molecular Conserved miR*		*Chromossomic Location*
Human	hsa-miR-29a 3p	UAGCACCAUCUGAAAUCGGUUA	7q32.3
		|||||||||||||||||||||	
	mmu-miR-29a 3p	UAGCACCAUCUGAAAUCGGUUA	
Mouse		|||||||||||||||||||||	6; 6 A3.3
Rat	rno-miR-29a 5p	UAGCACCAUCUGAAAUCGGUUA	4q22
Human	hsa-miR-29b 3p	UAGCACCAUUUGAAAUCAGUGUU	7q32.3
		|||||||||||||||||||||	
	mmu-miR-29b 3p	UAGCACCAUUUGAAAUCAGUGUU	
Mouse		|||||||||||||||||||||	6; 6 A3.3
Rat	rno-miR-29b 3p	UAGCACCAUUUGAAAUCAGUGUU	1
Human	hsa-miR-29c 5p	UUGUGACUAAAGUUUACCACGAU	1q32.2
		|||||||||||||||||||||	
	mmu-miR-29c 5p	UUGUGACUAAAGUUUACCACGAU	
Mouse		|||||||||||||||||||||	1; 1 H6
Rat	rno-miR-29c 5p	UUGUGACUAAAGUUUACCACGAU	13q27
Human	hsa-miR-206-3p	UGGAAUGUAAGGAAGUGUGUGG	6p12.2
		|||||||||||||||||||||	
	mmu-miR-206-3p	UGGAAUGUAAGGAAGUGUGUGG	
Mouse		|||||||||||||||||||||	1; 1 A4
Rat	rno-miR-206-3p	UGGAAUGUAAGGAAGUGUGUGG	9q13

Table 4. Seed sequence pairing when targeting IGF1 and its homologous.

mRNA-miRNA pairing			
HUMAN	901-908 of IGF1 3' UTR	5'UGUUUUUUAGUAUAAUGGUGCUA...	8mer
		|||||||	
	hsa-miR-29a-3p	3'AUUGGCUAAAGUCUACCACGAU	
	901-908 of IGF1 3' UTR	5'UGUUUUUUAGUAUAAUGGUGCUA...	8mer
		|||||||	
	hsa-miR-29b-3p	3'UUGUGACUAAAGUUUACCACGAU	
	901-908 of IGF1 3' UTR	5'UGUUUUUUAGUAUAAUGGUGCUA	8mer
		|||||||	
	hsa-miR-29c-3p	3'AUUGGCUAAAGUUUACCACGAU	
		|||||||	
MOUSE	3602-3609 of Igf1 3' UTR	5'CCAGCUACGCCAAUGUGGUGCUA...	8mer
		|||||||	
	mmu-miR-29a-3p	3'AUUGGCUAAAGUCUACCACGAU	
	3602-3609 of Igf1 3' UTR	5'CCAGCUACGCCAAUGUGGUGCUA...	8mer
		|||||||	
	mmu-miR-29b-3p	3'UUGUGACUAAAGUUUACCACGAU	
	3602-3609 of Igf1 3' UTR	5'CCAGCUACGCCAAUGUGGUGCUA...	8mer
		|||||||	
	mmu-miR-29c-3p	3'AUUGGCUAAAGUUUACCACGAU	
RAT	979-986 of Igf1 3' UTR	5'UUGUUUUUAGUACAAUGGUGCUA...	8mer
		|||||||	
	rno-miR-29a-3p	3'AUUGGCUAAAGUCUACCACGAU	
	979-986 of Igf1 3' UTR	5'UUGUUUUUAGUACAAUGGUGCUA...	8mer
		|||||||	
	rno-miR-29b-3p	3'UUGUGACUAAAGUUUACCACGAU	
	979-986 of Igf1 3' UTR	5'UUGUUUUUAGUACAAUGGUGCUA...	8mer
		|||||||	
	rno-miR-29c-3p	3'UUGUGACUAAAGUUUACCACGAU	

Table 5. Seed position miR-206 and BDNF.

mRNA-miRNA pairing			
Human	220-226 of BDNF 3' UTR	5'UAAAAAGUCUGCAUUACAUUCCU...	7mer-m8
		| | | | | | |	
	hsa-miR-206	3'GGUGUGUGAAGGAAUGUAAGGU	
Mouse	213-219 of Bdnf 3' UTR	5'UUAAAAGUCUGCAUUACAUUCCU...	7mer-m8
		| | | | | | |	
	mmu-miR-206-3p	3'GGUGUGUGAAGGAAUGUAAGGU	
Rat	214-220 of Bdnf 3' UTR	5'UUAAAAGUCUGCAUUACAUUCCU...	7mer-m8
		| | | | | | |	
	rno-miR-206-3p	3'GGUGUGUGAAGGAAUGUAAGGU	

Table 6. miRNA-IGF1 - Human Interaction validation experimentally.

Experimental Interaction						
miRNAS	***miRTarbase 2020***			***TarBase V.08***		
	Tissue	**Validation Method**	**Reference**	**Tissue**	**Validation Method**	**Reference**
miR-29a	Epididymis	Luciferase reporter assay qRTPCR Western blot	Ma et al. (2012)	Brain	HITS-CLIP	Boudreau et al. (2014)
miR-29b	-	-	-	Brain	HITS-CLIP	Boudreau et al. (2014)
miR-29c	-	-	-	Brain	HITS-CLIP	Boudreau et al. (2014)

(-) Ausence of Correlation data.

Table 7. miRNA-Igf1 - Mouse Interaction validation experimentally.

Experimental Interaction						
miRNAS	***miRTarbase 2020***			***TarBase V.08***		
	Tissue	***Validation Method***	***Reference***	***Tissue***	***Validation Method***	***Reference***
miR-29a	Brain	ELISA Luciferase repórter assay qRT-PCR	Fenn et al. (2013)	Embryo	Luciferase reporter qPCR	(Hand et al. 2012)
miR-29b	Brain	ELISA Luciferase repórter assay qRT-PCR	Fenn et al. (2013)	Muscle (C2C2)	HITS-CLIP	Zhang et al. (2014)
				Liver	HITS-CLIP	Schug et al. (2013)
miR-29c	-	-	-	-	-	-

(-) Ausence of Correlation data.

Reports from humans show miR-206 targets BDNF in gastric cancer cells, data validated by flow cytometry and qRT-PCR. In mouse skeletal muscle, C2C12 cells the interaction was confirmed by microarray and Luciferase assay in healthy conditions. In rat, miRTarbase confirmed the interaction between miR-206 and *Bdnf* in the brain by the use of rt-PCR, western blot, and luciferase assay.

3.3.3. Predicted miRNAs expression in T2DM skeletal muscle

The studies selected in GEO datasets contemplate *in vivo* and *in silico* data in humans and rats, all with microarray methodology. Was not found studies with the mouse in the database that contemplates the conditions searched. The miR-29a, miR-29b, miR-29c, and miR-206 presence

Table 8. mir-206-BDNF – Human, Mouse and Rat Interaction validation experimentally.

miRTarbase 2020								
Human			***Mouse***			***Rat***		
Tissue	***Validation Method***	***Reference***	***Tissue***	***Validation Method***	***Reference***	***Tissue***	***Validation Method***	***Reference***
Gastric Cancer Cells	Flow qRT-PCR	Ren et al. (2014)	C2C12	Luciferase reporter assay Microarray	Kim et al. (2006)	Brain	Luciferase reporter assay qRT-PCR ELISA Western Blot	(Sun et al.,2017)

Table 9. Analyses of miRNA Expression with T2DM Datasets from rat and human.

Expression Analyses				
miRNAS	***Rat datasets***		***Human datasets***	
	GSE68226 *in vivo*	**GSE26167 *in vivo***	**GSE68224 *in vivo***	**GSE86069 *in vitro***
miR-29a	*	X	*	*
miR-29b	*	X	X	*
miR-29c	*	X	*	*
miR-206	*	X	X	X

*Expressed in the study but not differentially.

or absence as differentially expressed in these studies' datasets are described in Table 9.

In human, dataset GSE68224 demonstrated that miR-29b and miR-206 were present as up-regulated in vastus lateralis skeletal muscle from prediabetics and T2DM subjects. However, from the same consortium, GSE68226 dataset contemplates rat subjects and found the expression of miR-29a, miR-29b, miR-29c, and miR-206 expressed in healthy and insulin resistance induced by high-fat diet conditions.

In the GSE86069 dataset, the expression of miR-29a, miR-29b, miR-29c, and miR-206 were elucidated as being present in all phases of muscle differentiation from healthy and T2DM human samples. Of the miRNAs candidates, only miR-206 was found with differential expression in the late muscle differentiation stage.

In rat dataset GSE26167, was reported that miR-29a, miR-29b, and miR-29c presented significant expression changes in T2DM rats when compared to control.

3.3.4. Network multiple analyses of miRNAs candidates and targets

The multiple analyses were performed with the human genome; the results demonstrated the network interactions with miR-29a, miR-29b, miR-29c, miR-206, and its submitted respective targets IGF1 and BDNF (Figure 4). Each gene and miRNA formed an isolated complex network with statistically and experimentally correlations to miRNA or target genes.

BDNF was correlated to miR-206 and other 48 miRNAs. While, IGF1 was correlated to a variety of 109 miRNAs, including the miR-29a, miR-29b, and miR-29c. The constructed network based on miRNAs candidates also demonstrated correlation to the respective target genes candidates (IGF1 and BDNF) as in the previous prediction evaluations. miR-29a-3p, were report to target 265 genes; miR-29b - 262, miR-29c - 255 and miR-206 - 122.

In Figure 5, are the top 10 pathways found in the network-generated enrichment analyses for GO: BP and diseases (Supplementary Material Table S3 and S4). The investigation demonstrated that the genes and miRNAs present in the gene-created cluster are highly related to regulation processes, mainly in metabolism. Of the miRNAs in the network, 22 were related to T2DM, including mir-29b and miR-206 in the evaluation for correlation with disease.

4. Discussion

Aggregated prediction approach unites different algorithms in view to increase the quality of results. Predictions considering only one type of tool tend to have few characteristics for carrying out the evaluation. Since each tool considers different features, this combined approach can provide a multifactorial evaluation and help the improvement of the prediction in view to improve the sensibility of the miRNAs selection.

Most of the predicted miRNAs for human IGF1 were members of the same family. miRNAs families are classified according to the miRNAs ancestor's derivation. Generally, members of the same family intend to perform roles in the same biological functions (Huang et al., 2009; Tapocik et al., 2014). The miR-29 family was highlighted in the evaluation for being recurrent in the three organisms. This agreement between them suggests that experimental genetic approaches in animals can be able to mimic gene modulation results in humans.

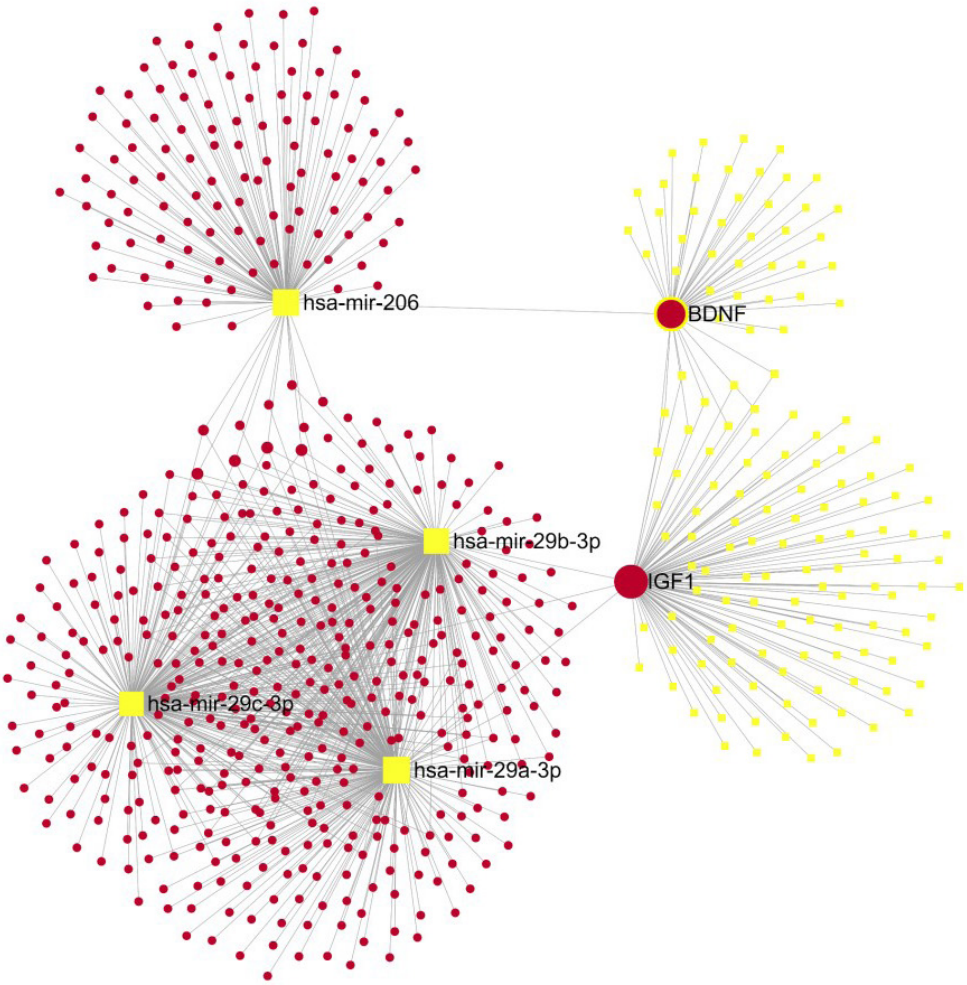

Figure 4. Network interaction miRNA-mRNA generate cluster.

Among the miRNAs predicted to target BDNF genes, rat presented a reduced number, which was a consequence of the absence of results from microT CDS tool. This result is befitting of the small number of rat cataloged miRNAs in miRBase, where are 496 (Kozomara et al., 2019). miR-206 targets BDNF genes from human, mouse, and rat predictions and were also predicted to target *Igf1* in the mouse. Thus, this data points to miRNA multiple target functioning, which endorses the need for specific approaches to predict the best seed binding affinity and specificity (Elton and Yalowich, 2015).

The convergency found in the miRNAs candidates in number and sequence suggests that the functioning of these molecules also can be similar due to the high evolutionary conservation between the studied animal models. Most of the RNA sequences are conserved through beings with the same direct ancestor. In this context, mammals use to present close molecular relationships also in miRNAs. The miRNA repression depends on the seed binding position, where the interaction happens with a match between 7 nucleotides. The most common targeting sites are 6mer, 7 mer-m8, and 8mer (Penso-Dolfin et al., 2018). Our analyses showed that the miRNAs found fit these criteria.

The miR-29 family showed high potential in targeting IGF1 in animal models studied. This interaction was strongly reported in the brain of human and mouse, causing a decrease in gene expression and, consequently, it is functioning (Fenn et al., 2013; Boudreau et al., 2014). miR-29b had many reports between the searched miR-29 family members. In mouse, this miRNA was identified to target *Igf1* gene in a study with C2C12 cells of mitochondrial protein synthesis during muscle myogenesis, where miR-29b was identified as downregulated in myoblast and myocytes in healthy conditions (Zhang et al., 2014).

In mouse liver regeneration, miR-29b is downregulated, while, studies recognized it in the opposite process, having an upregulation profile in liver disease pathogenesis. Thus, there is also suggested that in T2DM condition, it has some effects on non-alcoholic fatty liver disease (NAFLD). It was reported that NAFLD is prevalent in 56,67% of T2DM patients (Schug et al., 2013; Dharmalingam and Yamasandhi, 2018).

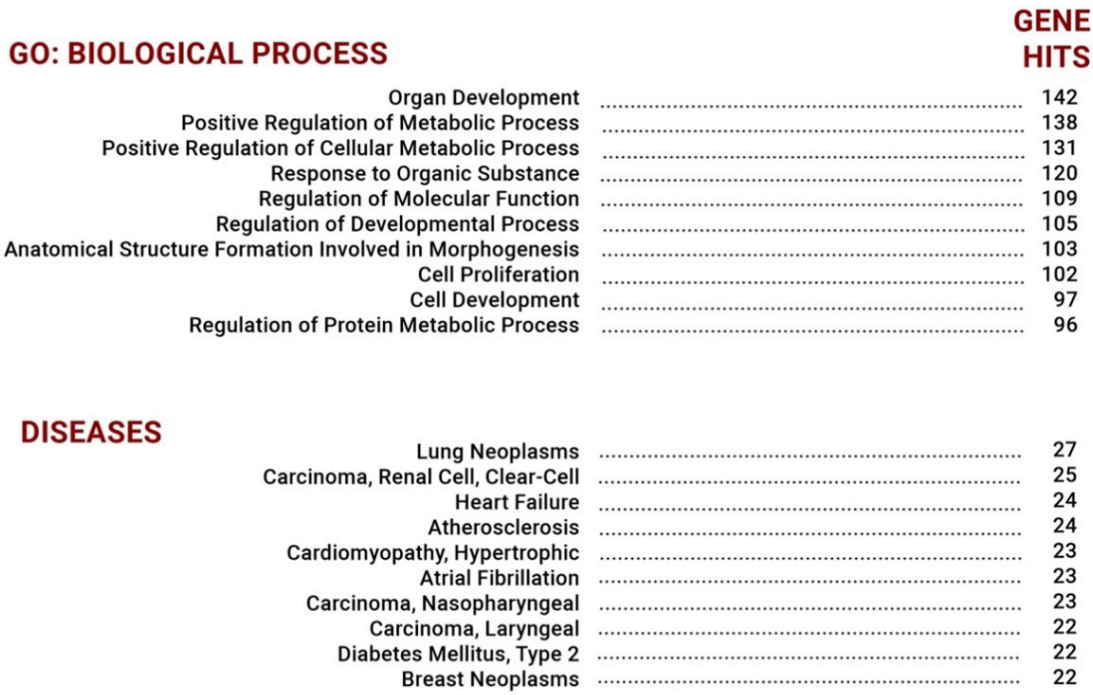

Figure 5. Top 10 enriched terms from GO Biological Process and Diseases.

For human organisms, the miR-206 – BDNF interaction is experimentally validated in gastric cancer, where the miRNA was up-regulated and the gene repressed (Ren et al., 2014). The "myomir" miR-206 is present in muscle differentiation from C2C12 lineage cells and is predicted to target *Bdnf* (Kim et al., 2006). In vitro approach confirmed that miR-206 suppressed *Bdnf* in C2C12 by rt-PCR, ELISA, and Northern Blot, validating the role of miR-206 in muscle differentiation signaling (Miura et al., 2012).

In the diabetes context, the datasets selected showed in rat GSE68226 dataset results insulin resistance induced by high-fat diet did not present differential expression of miR-29b, while, in prediabetics human and T2DM (GSE68224) this miRNA were differentially expressed (Latouche et al., 2016). This discrepancy may be due to insulin resistance conditions in rats since it is a predisposition step for diabetes, so at this state different miRNAs can be expressed (Taylor, 2012).

In the GSE26167 study, between the four candidates, miR-29a was relevant for being highly up-regulated in T2DM, which was later validated by rtPCR. The miR-29a expression profile was also described in human blood from T2DM subjects from the same cohort study (GSE21321) (Karolina et al., 2011).

GSE86069 investigated the stages of muscle proliferation and differentiation in human muscle healthy and T2DM donors. miR-206 was found in this context, with a reduced expression signature through the muscle differentiation stage in T2DM, and this finding was correlated to the reduction in myogenesis in T2DM cells (Henriksen et al., 2017).

In view to emphasize the potential therapeutic application for the human genome, the miRNA found and targets were submitted to network analyses. The results confirmed the interaction between miRNA and the target gene and also demonstrate that are a large number of interaction possibilities and physiological processes related. This result remits the importance of direct and indirect miRNA-mRNA interaction identification in the selection of functional mechanisms, excluding the interactions that are only biological noise (Cloonan, 2015). All the interactions disposed of in the network were obtained by the tool for construction are based on well-known prediction tools and data experimentally validated with also a well-annotated database (Edgar et al., 2002).

4. Conclusion

The evaluations of the presented study brought data that evidenced through *in silico* investigation a strong targeting relationship between the miRNAs mir-29 family and mir-206 with IGF1 and BDNF. Furthermore, the investigations showed high similarity and conservation between the related interactions in the three organisms studied, demonstrating a potential for reproducing these findings in different models. The evaluation of *in vitro* and *in vivo* studies in databases also corroborates these findings in several different tissues and also in the T2DM context, in which was observed that the miRNAs found were differentially expressed. The network evaluation results included metabolic paths and T2DM. Nevertheless,

these results point to miRNA-mRNA strong interaction for future biotechnological prospects.

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References

AGARWAL, V., BELL, G.W., NAM, J.W. and BARTEL, D.P., 2015. Predicting effective microRNA target sites in mammalian mRNAs. *eLife*, vol. 4, e05005. http://dx.doi.org/10.7554/eLife.05005. PMid:26267216.

BOUDREAU, R.L., JIANG, P., GILMORE, B.L., SPENGLER, R.M., TIRABASSI, R., NELSON, J.A., ROSS, C.A., XING, Y. and DAVIDSON, B.L., 2014. Transcriptome-wide discovery of microRNA binding sites in Human Brain. *Neuron*, vol. 81, no. 2, pp. 294-305. http://dx.doi.org/10.1016/j.neuron.2013.10.062. PMid:24389009.

CHANG, L., ZHOU, G., SOUFAN, O. and XIA, J., 2020. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Research*, vol. 48, no. W1, pp. W244-W251. http://dx.doi.org/10.1093/nar/gkaa467. PMid:32484539.

CHEN, Y. and WANG, X., 2020. MiRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Research*, vol. 48, no. D1, pp. D127-D131. http://dx.doi.org/10.1093/nar/gkz757. PMid:31504780.

CLOONAN, N., 2015. Re-thinking miRNA-mRNA interactions: intertwining issues confound target discovery. *BioEssays*, vol. 37, no. 4, pp. 379-388. http://dx.doi.org/10.1002/bies.201400191. PMid:25683051.

DHARMALINGAM, M. and YAMASANDHI, P.G., 2018. Nonalcoholic fatty liver disease and Type 2 diabetes mellitus. *Indian Journal of Endocrinology and Metabolism*, vol. 22, no. 3, pp. 421-428. http://dx.doi.org/10.4103/ijem.IJEM_585_17. PMid:30090738.

DIANA LAB, 2021 [viewed 24 September 2021]. *microRNA Research* [online]. Available from: http://www.microrna.gr

DIANA TOOLS, 2021 [viewed 24 September 2021]. *TarBase v.8* [online]. Available from: http://www.microrna.gr/tarbase

EDGAR, R., DOMRACHEV, M. and LASH, A.E., 2002. Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Research*, vol. 30, no. 1, pp. 207-210. http://dx.doi.org/10.1093/nar/30.1.207. PMid:11752295.

ELTON, T.S. and YALOWICH, J.C., 2015. Experimental procedures to identify and validate specific mRNA targets of miRNAs. *EXCLI Journal*, vol. 14, pp. 758-790. http://dx.doi.org/10.17179/excli2015-319. PMid:27047316.

FENN, A.M., SMITH, K.M., LOVETT-RACKE, A.E., GUERAU-DE-ARELLANO, M., WHITACRE, C.C. and GODBOUT, J.P., 2013. Increased micro-RNA 29b in the aged brain correlates with the reduction of insulin-like growth factor-1 and fractalkine ligand. *Neurobiology of Aging*, vol. 34, no. 12, pp. 2748-2758. http://dx.doi.org/10.1016/j.neurobiolaging.2013.06.007. PMid:23880139.

FUJINAMI, A., OHTA, K., OBAYASHI, H., FUKUI, M., HASEGAWA, G., NAKAMURA, N., KOZAI, H., IMAI, S. and OHTA, M., 2008. Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: relationship to glucose metabolism and biomarkers of insulin resistance. *Clinical Biochemistry*, vol. 41, no. 10-11, pp. 812-817. http://dx.doi.org/10.1016/j.clinbiochem.2008.03.003. PMid:18402781.

HAND, N.J., HORNER, A.M., MASTER, Z.R., BOATENG, L.A., LEGUEN, C., UVAYDOVA, M. and FRIEDMAN, J.R., 2012. MicroRNA profiling identifies miR-29 as a regulator of disease-associated pathways in experimental biliary atresia. *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 2, pp. 186–192. DOI 10.1097/MPG.0b013e318244148b.

HEBERLE, H., MEIRELLES, V.G., SILVA, F.R., TELLES, G.P. and MINGHIM, R., 2015. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics*, vol. 16, no. 1, pp. 169. http://dx.doi.org/10.1186/s12859-015-0611-3. PMid:25994840.

HENRIKSEN, T.I., DAVIDSEN, P.K., PEDERSEN, M., SCHULTZ, H.S., HANSEN, N.S., LARSEN, T.J., VAAG, A., PEDERSEN, B.K., NIELSEN, S. and SCHEELE, C., 2017. Dysregulation of a novel miR-23b/27b-p53 axis impairs muscle stem cell differentiation of humans with type 2 diabetes. *Molecular Metabolism*, vol. 6, no. 7, pp. 770-779. http://dx.doi.org/10.1016/j.molmet.2017.04.006. PMid:28702332.

HUANG, H.Y., LIN, Y.C., LI, J., HUANG, K.Y., SHRESTHA, S., HONG, H.C., TANG, Y., CHEN, Y.G., JIN, C.N., YU, Y., XU, J.T., LI, Y.M., CAI, X.X., ZHOU, Z.Y., CHEN, X.H., PEI, Y.Y., HU, L., SU, J.J., CUI, S.D., WANG, F., XIE, Y.Y., DING, S.Y., LUO, M.F., CHOU, C.H., CHANG, N.W., CHEN, K.W., CHENG, Y.H., WAN, X.H., HSU, W.L., LEE, T.Y., WEI, F.X. and HUANG, H.D., 2020. miRTarBase 2020: updates to the experimentally validated microRNA-target interaction database *Nucleic Acids Research*, vol. 48, no. D1, pp. D148-D154. http://dx.doi.org/10.1093/nar/gkz896. PMid:31647101.

HUANG, W., SHERMAN, B.T. and LEMPICKI, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols*, vol. 4, no. 1, pp. 44-57. http://dx.doi.org/10.1038/nprot.2008.211. PMid:19131956.

INTERACTIVENN [online], 2021 [viewed 24 September 2021]. Available from: http://www.interactivenn.net/

JUNG, H.J. and SUH, Y., 2015. Regulation of IGF -1 signaling by microRNAs. *Frontiers in Genetics*, vol. 5, pp. 472. http://dx.doi.org/10.3389/fgene.2014.00472. PMid:25628647.

KARAGKOUNI, D., PARASKEVOPOULOU, M.D., CHATZOPOULOS, S., VLACHOS, I.S., TASTSOGLOU, S., KANELLOS, I., PAPADIMITRIOU, D., KAVAKIOTIS, I., MANIOU, S., SKOUFOS, G., VERGOULIS, T., DALAMAGAS, T. and HATZIGEORGIOU, A.G., 2018. DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA-gene interactions. *Nucleic Acids Research*, vol. 46, no. D1, pp. D239-D245. http://dx.doi.org/10.1093/nar/gkx1141. PMid:29156006.

KAROLINA, D.S., ARMUGAM, A., TAVINTHARAN, S., WONG, M.T.K., LIM, S.C., SUM, C.F. and JEYASEELAN, K., 2011. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. *PLoS One*, vol. 6, no. 8, pp. e22839. http://dx.doi.org/10.1371/journal.pone.0022839. PMid:21829658.

KHARROUBI, A.T. and DARWISH, H.M., 2015. Diabetes mellitus: the epidemic of the century. *World Journal of Diabetes*, vol. 6, no. 6, pp. 850-867. http://dx.doi.org/10.4239/wjd.v6.i6.850. PMid:26131326.

KIM, H.K., LEE, Y.S., SIVAPRASAD, U., MALHOTRA, A. and DUTTA, A., 2006. Muscle-specific microRNA miR-206 promotes muscle differentiation. *The Journal of Cell Biology*, vol. 174, no. 5, pp. 677-687. http://dx.doi.org/10.1083/jcb.200603008. PMid:16923828.

KIM, M. and ZHANG, X., 2019. The profiling and role of miRNAs in diabetes mellitus. *Journal of Diabetes and Clinical Research*, vol. 1, no. 1, pp. 5-23. PMid:32432227.

KOZOMARA, A., BIRGAOANU, M. and GRIFFITHS-JONES, S., 2019. MiRBase: from microRNA sequences to function. *Nucleic Acids Research*, vol. 47, no. D1, pp. D155-D162. http://dx.doi.org/10.1093/nar/gky1141. PMid:30423142.

KRÜTZFELDT, J., RAJEWSKY, N., BRAICH, R., RAJEEV, K.G., TUSCHL, T., MANOHARAN, M. and STOFFEL, M., 2005. Silencing of microRNAs in vivo with "antagomirs.". *Nature*, vol. 438, no. 7068, pp. 685-689. http://dx.doi.org/10.1038/nature04303. PMid:16258535.

LATOUCHE, C., NATOLI, A., REDDY-LUTHMOODOO, M., HEYWOOD, S.E., ARMITAGE, J.A. and KINGWELL, B.A., 2016. MicroRNA-194 modulates glucose metabolism and its skeletal muscle expression is reduced in diabetes. *PLoS One*, vol. 11, no. 5, e0155108. http://dx.doi.org/10.1371/journal.pone.0155108. PMid:27163678.

MA, W., XIE, S., NI, M., HUANG, X., HU, S., LIU, Q., LIU, A., ZHANG, J. and ZHANG, Y., 2012. MicroRNA-29a Inhibited Epididymal Epithelial Cell Proliferation by Targeting Nuclear Autoantigenic Sperm Protein (NASP). *Journal of Biological Chemistry*, vol. 287, no. 13, pp. 10189–10199. DOI 10.1074/jbc.M111.303636.

MCDONALD, A., WILLIAMS, R.M., REGAN, F.M., SEMPLE, R.K. and DUNGER, D.B., 2007. IGF-I treatment of insulin resistance. *European Journal of Endocrinology*, vol. 157, suppl. 1, pp. S51-S56. http://dx.doi.org/10.1530/EJE-07-0271. PMid:17785698.

MIRDB [online], 2021 [viewed 24 September 2021]. Available from: http://mirdb.org/

MIRNET [online], 2021 [viewed 24 September 2021]. Available from: https://www.mirnet.ca/

MIRTARBASE [online], 2021 [viewed 24 September 2021]. Available from: http://miRTarBase.cuhk.edu.cn/

MIURA, P., AMIROUCHE, A., CLOW, C., BÉLANGER, G. and JASMIN, B.J., 2012. Brain-derived neurotrophic factor expression is repressed during myogenic differentiation by miR-206. *Journal of Neurochemistry*, vol. 120, no. 2, pp. 230-238. http://dx.doi.org/10.1111/j.1471-4159.2011.07583.x. PMid:22081998.

NATIONAL CENTER FOR BIOTECHNOLOGY – NCBI, 2021 [viewed 24 September 2021]. *Gene Expression Omnibus (GEO)* [online]. Bethesda: NCBI. Available from: https://www.ncbi.nlm.nih.gov/geo

NUMAKAWA, T., RICHARDS, M., ADACHI, N., KISHI, S., KUNUGI, H. and HASHIDO, K., 2011. MicroRNA function and neurotrophin BDNF. *Neurochemistry International*, vol. 59, no. 5, pp. 551-558. http://dx.doi.org/10.1016/j.neuint.2011.06.009. PMid:21723895.

O'BRIEN, J., HAYDER, H., ZAYED, Y. and PENG, C., 2018. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology*, vol. 9, pp. 402. http://dx.doi.org/10.3389/fendo.2018.00402. PMid:30123182.

PARASKEVOPOULOU, M.D., GEORGAKILAS, G., KOSTOULAS, N., VLACHOS, I.S., VERGOULIS, T., RECZKO, M., FILIPPIDIS, C., DALAMAGAS, T. and HATZIGEORGIOU, A.G., 2013. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. *Nucleic Acids Research*, vol. 41, no. W1, pp. W169. http://dx.doi.org/10.1093/nar/gkt393. PMid:23680784.

PENSO-DOLFIN, L., MOXON, S., HAERTY, W. and DI PALMA, F., 2018. The evolutionary dynamics of microRNAs in domestic mammals. *Scientific Reports*, vol. 8, no. 1, pp. 17050. http://dx.doi.org/10.1038/s41598-018-34243-8. PMid:30451897.

PHIELIX, E. and MENSINK, M., 2008. Type 2 diabetes mellitus and skeletal muscle metabolic function. *Physiology & Behavior*, vol. 94, no. 2, pp. 252-258. http://dx.doi.org/10.1016/j.physbeh.2008.01.020. PMid:18342897.

REN, J., HUANG, H., GONG, Y., YUE, S., TANG, L. and CHENG, S.Y., 2014. MicroRNA-206 suppresses gastric cancer cell growth and metastasis. *Cell & Bioscience*, vol. 4, no. 1, pp. 26. http://dx.doi.org/10.1186/2045-3701-4-26. PMid:24855559.

ROZANSKA, O., URUSKA, A. and ZOZULINSKA-ZIOLKIEWICZ, D., 2020. Brain-derived neurotrophic factor and diabetes. *International Journal of Molecular Sciences*, vol. 21, no. 3, pp. 841. http://dx.doi.org/10.3390/ijms21030841. PMid:32012942.

SCHUG, J., MCKENNA, L.B., WALTON, G., HAND, N., MUKHERJEE, S., ESSUMAN, K., SHI, Z., GAO, Y., MARKLEY, K., NAKAGAWA, M., KAMESWARAN, V., VOUREKAS, A., FRIEDMAN, J.R., KAESTNER, K.H. and GREENBAUM, L.E., 2013. Dynamic recruitment of microRNAs to their mRNA targets in the regenerating liver. *BMC Genomics*, vol. 14, no. 1, pp. 264. http://dx.doi.org/10.1186/1471-2164-14-264. PMid:23597149.

SINAIKO, A.R. and CAPRIO, S., 2012. Insulin resistance. *The Journal of Pediatrics*, vol. 161, no. 1, pp. 11-15. http://dx.doi.org/10.1016/j.jpeds.2012.01.012. PMid:22336575.

SUDA, K., MATSUMOTO, R., FUKUOKA, H., IGUCHI, G., HIROTA, Y., NISHIZAWA, H., BANDO, H., YOSHIDA, K., ODAKE, Y., TAKAHASHI, M., SAKAGUCHI, K., OGAWA, W. and TAKAHASHI, Y., 2016. The influence of type 2 diabetes on serum GH and IGF-I levels in hospitalized Japanese patients. *Growth Hormone & IGF Research*, vol. 29, pp. 4-10. http://dx.doi.org/10.1016/j.ghir.2016.03.002. PMid:27060213.

SUN, W., ZHANG, L. and LI, R., 2017. Overexpression of miR-206 ameliorates chronic constriction injury-induced neuropathic pain in rats via the MEK/ERK pathway by targeting brain-derived neurotrophic factor. *Neuroscience Letters*, vol. 646, pp. 68–74. DOI 10.1016/j.neulet.2016.12.047.

TAPOCIK, J.D., BARBIER, E., FLANIGAN, M., SOLOMON, M., PINCUS, A., PILLING, A., SUN, H., SCHANK, J.R., KING, C. and HEILIG, M., 2014. MicroRNA-206 in rat medial prefrontal cortex regulates BDNF expression and alcohol drinking. *The Journal of Neuroscience*, vol. 34, no. 13, pp. 4581-4588. http://dx.doi.org/10.1523/JNEUROSCI.0445-14.2014. PMid:24672003.

TARGETSCAN [online], 2021 [viewed 24 September 2021]. Available from: http://www.targetscan.org

TAYLOR, R., 2012. Insulin resistance and type 2 diabetes. *Diabetes*, vol. 61, no. 4, pp. 778-779. http://dx.doi.org/10.2337/db12-0073. PMid:22442298.

VISHNOI, A. and RANI, S., 2017. MiRNA biogenesis and regulation of diseases: an overview. *Methods in Molecular Biology*, vol. 1509, pp. 1-10. http://dx.doi.org/10.1007/978-1-4939-6524-3_1. PMid:27826912.

ZHANG, X., ZUO, X., YANG, B., LI, Z., XUE, Y., ZHOU, Y., HUANG, J., ZHAO, X., ZHOU, J., YAN, Y., ZHANG, H., GUO, P., SUN, H., GUO, L., ZHANG, Y. and FU, X.D., 2014. MicroRNA directly enhances mitochondrial translation during muscle differentiation. *Cell*, vol. 158, no. 3, pp. 607-619. http://dx.doi.org/10.1016/j.cell.2014.05.047. PMid:25083871.

Supplementary material

Table S1 – List of miRNA predictions using IGF1 as query for human, rat and mouse
Table S2 – List of miRNA predictions using BDNF as the query for human, rat, and mouse
Table S3 – List generate in miRNet Enrichment Results for GO BP
Table S4 – List generate in miRNet Enrichment Results for diseases
This material is available as part of the online article from https://www.scielo.br/j/bjb