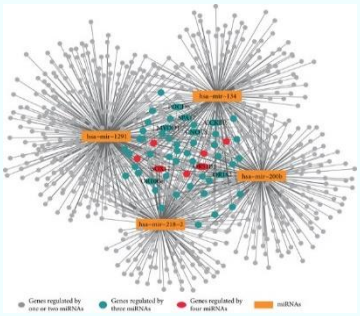
JOURNAL OF CLINICAL SURGERY AND RESEARCH

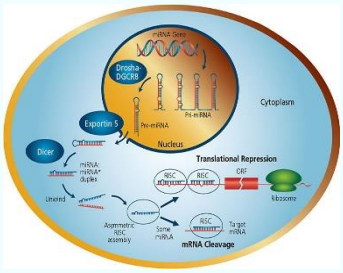
The NS-3 Mixture of δ-Tocotrienol, Vitamin D_3 and Resveratrol Modulates Gene Expression of Several Novel MicroRNAs Identified by Transcriptomic Analyses in People with Type 2 Diabetes

Corresponding Author: Asaf A. Qureshi



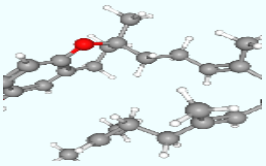
MicroRIBONUCLEIC ACIDS (miRNAs)

MicroRNAs are small non-coding RNA molecules present in cells of humans, animals, and plants, and play an important role by regulating of human genome in a number, of biological processes (proliferation, differentiation, development, and apoptosis). A single miRNA may act on multiple targets and results in destruction or suppression of translation in multiple messengerRNA (mRNA), or single mRNA which is regulated by multiple miRNAs and has vast regulatory potential.



δ-TOCOTRIENOL

δ-Tocotrienol is a form of vitamin E that belongs to tocotrienols family. Tocotrienols and tocopherols constitute two main groups of vitamin E compounds.

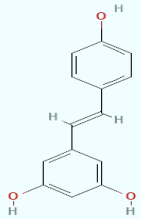


VITAMIN D_3

Vitamin D_3 (cholecalciferol) is a crucial nutrient that plays various roles in the body, such as calcium absorption and bone health.

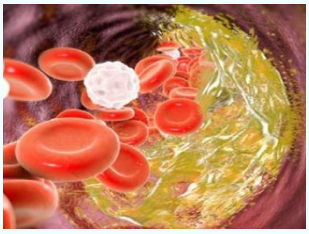
RESVERATROL (Nutritional Supplement-NS-3)

Resveratrol is a natural compound found in certain plants, including the skin of red grapes, blueberries, raspberries, and peanuts. It belongs to a group of plant compounds called polyphenols and is known for its antioxidant properties.



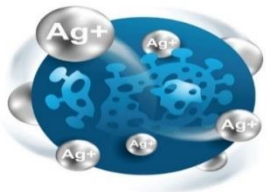
ANTI-CHOLESTEROLEMIC AGENTS

Anti-cholesterolemic agents' lower cholesterol in the body. Cholesterol is a fat, found in the blood, involves in many body functions. The elevated level of cholesterol (particularly, LDL-cholesterol) can increase the risk of cardiovascular disease.



ANTI-INFLAMMATORY AGENTS

Anti-inflammatory agents reduce inflammation in the body. It is a natural response by immune system to injury or infection. Chronic inflammation can cause cardiovascular disease, diabetes, arthritis, and cancer.



Authored by

Asaf A Qureshi[1], Dilshad A. Khan[2], Wajiha Mahjabeen[2], Nilofer Qureshi[1,3], Mrunal K. Dehankar[4] and Danial P Heruth[5]**

[1]Department of Biomedical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA.

[2]Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), National University of Medical Sciences, Rawalpindi, 64000, Pakistan.

[3]Pharmacology/Toxicology, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA.

[4]Mayo Clinic, 200 First Street SW, Rochester, Minnesota, 55905, USA.

[5]Division of Experimental and Translational Genetics, Department of Pediatrics, Children's Mercy Hospital, 2401 Gillham Road, Kansas City, MO. 64108, USA.

Published Date

04 April, 2024

Published in the Journal of

Clinical Surgery and Research

Auctores Publishing, LLC

16192 Coastal Highway

Lewes, DE 19958,

United States of America.

AUCTORES
Globalize your Research

Journal of Clinical Surgery and Research

Asaf A. Qureshi**

Open Access

Research Article

*Re-Print: The NS-3 Mixture of δ-Tocotrienol, Vitamin D_3 and Resveratrol Modulates Gene Expression of Several Novel MicroRNAs Identified by Transcriptomic Analyses in People with Type 2 Diabetes

Asaf A Qureshi[1], Dilshad A. Khan[2], Wajiha Mahjabeen[2], Nilofer Qureshi[1,3], Mrunal K. Dehankar[4], and Danial P Heruth[5]**

[1] Department of Biomedical Science, School of Medicine, University of Missouri-Kansas City, 2411 Holmes Street, Kansas City, MO 64108, USA.

[2] Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), National University of Medical Sciences, Rawalpindi, 64000, Pakistan.

[3] Pharmacology/Toxicology, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA.

[4] Mayo Clinic, 200 First Street SW, Rochester, Minnesota, 55905, USA.

[5]Division of Experimental and Translational Genetics, Department of Pediatrics, Children's Mercy Hospital, 2401 Gillham Road, Kansas City, MO. 64108, USA.

**Corresponding Author: Asaf A. Qureshi, Ph.D. Department of Biomedical Science, 2411 Holmes Street, School of Medicine, University of Missouri, Kansas City, MO 64108, USA. E-mail: qureshia@umsystem.edu

Received Date: 14 March, 2024 | Accepted Date: 22 March, 2024 | Published Date: 04 April, 2024

Citation: Asaf A Qureshi, Dilshad A. Khan, Wajiha Mahjabeen, Nilofer Qureshia, Mrunal K. Dehankar, (2024), *Re-Print: The NS-3 Mixture of δ-Tocotrienol, Vitamin D_3 and Resveratrol Modulates Gene Expression of Several Novel MicroRNAs Identified by Transcriptomic Analyses in People with Type 2 Diabetes, *Journal of Clinical Surgery and Research*, 5(3); **DOI:10.31579/2768-2757/118**

Abstract:

Aims: Type 2 diabetes mellitus is due to hyperglycemia, therefore fasting glucose and glycosylated hemoglobin (HbA1c) levels are used as biomarkers to determine onset of diabetes. RT-PCR estimation of pooled total mRNAs of EDTA treated whole blood (plasma) obtained after the treatment of NS-3 mixture of δ-tocotrienol, vitamin D_3, resveratrol of people with type 2 diabetes mellitus (T2DM) for 24 weeks, showed significant down-regulation of gene expression of several diabetes biomarkers (IRS-1, SOD-2, GCKR, IGFBP-2) and cytokines (IL-4, IL-6) as compared to pre-treatment values. Present study investigates the effectiveness of NS-3 on gene expression of mRNAs, miRNAs, and paired mRNA-miRNA in people with T2DM.

Methods: Present study is an extension of a randomized placebo controlled double-blinded clinical trial of T2DM (n = 56/group) given two capsules/d of cellulose/olive oil (placebo), or NS-3 for 24-weeks. Pure mRNAs and miRNAs of plasma of pre-dose versus post-dose of NS-3 treated samples were analyzed by next generation sequencing (NGS). Data was uploaded into "Ingenuity Pathways Analyses".

Results: A total of 4000 genes are considered significant, based on > 2-fold gene expression changes. Out of which 1373 genes are significantly differentially expressed in pre-dose vs post-dose (P < 0.02) samples, 20 are up-regulated and 27 are down-regulated of NS-3 treated RNAs of T2DM. Gene expression of up-regulated miR-29b-3p modulates (GLUT4, insulin resistance), miR-624-5p (nephropathy biomarker), miR-361-5p (chronic inflammation), miR-130a-3p (glucose metabolism, insulin secretion), miR-3912-3p (lipid metabolism), and miR-11401 (cellular transcription). The miR-374c-5p (insulin resistance), miR-4326 (HbA1c level)), miR-874-3p (β-cell function) are down-regulated of NS-3 treated people with T2DM. Whereas messengerR-ML-1621513 (oxidative/stress), mR-CTD-2349P217 (insulin-mediated glucose-uptake), are up-regulated, and mR-CTC-246B1810 (β-cell/biology) are down-regulated in T2DM after NS-3 treatment. Venn diagrams have established genetic regulatory network images and canonical signaling pathways for mRNA, miRNA, and paired mRNA-miRNA of gene expression profiles of pre-dose vs post-dose of NS-3 treatment group.

Conclusions: The NS-3 treatment of people with T2DM indicates up- or down-regulation of several new miRNAs (miR-29b-3p, miR-624-5p, miR-361-5p, miR-130a-3p, miR-3912-3p, miR-374c-5p, miR-4326 [HbA1c], miR-1247-3p, miR-874-5p) which

may be used to identify onset of T2DM. Overexpression of mRNA-AL1621513 indicates oxidative stress in people with T2DM, resulting in complications of diabetes (neuropathy, retinopathy, and stroke).

keywords: T2DM; δ -tocotrienol; vitamin D_3; resveratrol; miRNA-29b-3p; mRNA-AL1621513.

Introduction

We have recently reported results of RT-PCR estimation of pooled total mRNAs obtained after the treatment of NS-3 mixture of δ -tocotrienol, vitamin D_3 and resveratrol **(Figure 1)** of people with type 2 diabetes mellitus (T2DM), which showed significant ($P < 0.001$) down-regulation of gene

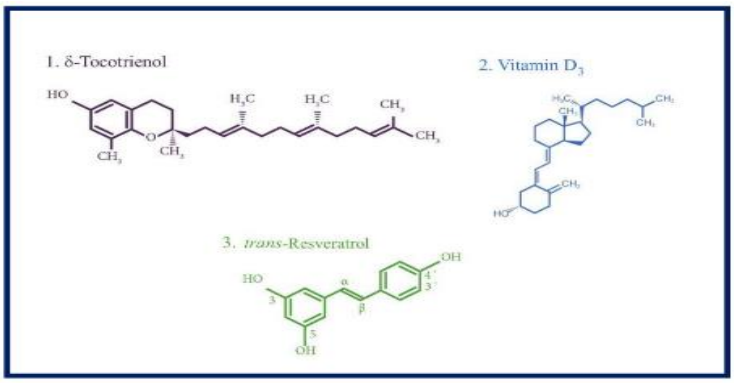


Figure 1: Structures of various ingredients

expression of several diabetes biomarkers (IRS-1, SOD-2, GCKR, IGFBP-2) and cytokines (IL-4, IL-6) as compared to pre-treatment values [1]. These results prompted us to carry out the effect of NS-3 treated RNAs of people with T2DM on mRNA- and miRNA-sequencing by next-generation sequencing (NGS). Diabetes mellitus is a metabolic disorder characterized by hyperglycemia which is either due to deficiency of insulin production or insulin resistance (IR) or both [2]. The risk factors like impaired fasting glucose, glucose tolerance and glycosylated hemoglobin (HbA1c) are normally used to diagnose the onset of diabetes mellitus, which are not sensitive enough and thus do not have the definitive predictive values [3]. Therefore, a new set of biomarkers are required that can detect the progression of organ damage or diagnose the complications of disease even before the disease become clinically evident. Recently, the role of microRNAs (miRNAs) is gaining importance in diagnosis diseases in humans [4].

MicroRNAs are small non-coding RNA molecules present in cells from humans, animals, and plants, and that play an important role by regulating 60% of human genome in a number, of biological processes such as proliferation, differentiation, development, and apoptosis [4]. It functions either at transcriptional and post-transcriptional levels and controls the production of specific gene products (protein) [5]. A single miRNA may act on multiple targets and results in destruction or suppression of translation in multiple messenger RNA (mRNA), or single mRNA which is regulated by multiple miRNAs provides vast regulatory potential. In order, to alter the protein synthesis, miRNA forms the base pairing (2-8 (average 7) nucleotides at 5' end of miRNA with complementary sequences within the 3'UTR of mRNA. This binding of miRNA with mRNA is either in the form of full or partial complement homology [6]. The complete homology dependent mRNA degradation is more common in lower vertebrates whereas in mammals, partial complement homology results in suppression of translation process of mRNA [6].

A number of studies have described miRNAs play an important role in insulin secretion, glucose homeostasis and adipocytes differentiation, particularly, miR-21, miR-126, miR-146a were the most altered miRNAs among healthy and people with T2DM [5-8]. The risk of developing T2DM increased in individuals having highest circulatory miRNA-146. A recent study found a positive association of miRNA-146 levels with heme oxygenase-1, an enzyme having potent anti-oxidative, anti-inflammatory, and anti-proliferative effects and negative association with homeostasis of HOMA-β [7]. The results of another study using peripheral blood mononuclear cells (PBMCs) from people with diabetes and normal individuals, the gene expression of miRNA-146 was significantly decreased in people with diabetes as compared to non-diabetic control individuals. While the plasma levels of IL-6 and TNF-α significantly increased in these individuals. The study also showed the association of decreased levels of miRNA-146a with insulin resistance, decreased glucose control, increased expression of TRAF-6, and NF-κB in people with T2DM [8]. Therefore, hyperglycemia may be responsible to down-regulation of plasma miRNA-146a expression in people with T2DM, and decreased gene expression of miRNA-146a causes the elevated inflammatory response and may contribute to impairment in diabetic wound healing [9,10]. The present investigation was undertaken to explore the role of mRNAs, miRNAs, and their interaction in people with T2DM, which may help to determine the ability of miRNAs for early detection of onset of diabetes.

Materials and Methods

Materials:

The 70% tocotrienols mixture (typical composition 90% δ-tocotrienol and 10% γ-tocotrienol) purified from annatto seeds was purchased from American River Nutrition, Inc. (Hadley, MA, USA); *trans*-Resveratrol from "Mega Resveratrol" (60 Newton Road # 32 Danbury CT, USA) and Vitamin D_3 purchased from Piping Rock, NY. The purification of 70% δ-tocotrienol to 98%, and capsulation of mixture of NS-3 of δ -tocotrienol (125 mg), resveratrol (125 mg), vitamin D_3 (125 μg [5000 IU]) = 250.125 mg/capsule, placebo capsule (125.125 mg cellulose + 125 mg olive oil = 250.125 mg) has been described earlier [1].

Experimental:

Extraction of total mRNAs and miRNAs from EDTA treated blood (Plasma) after treatment with a mixture of NS-3 to people with T2DM.

The details of study design of a randomized placebo controlled double-blinded trial has been reported in our earlier publication [1]. In short, the

study protocol was registered with WHO regional office in Asia (World Health Organization Sri Lanka Clinical Trials Registry, Sri Lanka Center; srilankactr@gmail.com), after ethical approval by the Institutional Review Board of Armed forces institute of pathology (AFIP), Rawalpindi, Pakistan. The registry number was SLCTR/2018/019, dated 6.21.2018. All participants of the study signed an informed consent form before start of the study. People with T2DM (***n*** = 112) aged >30 years in this project were screened at Armed forces Institute of Pathology (AFIP), Rawalpindi, Pakistan. Their clinical history and general physical examination of each patient was recorded [1]. The baseline venous blood samples (12 h fast, 7.00 - 9.00 am) were collected, then study participants were randomly divided into two groups. The participants of placebo group A (n = 56) were provided two capsules/d of AMR-1 and group B (***n*** = 56) two capsules/d of a mixture of NS-3 for 24-weeks. The capsules in each group were administered one capsule after breakfast and second after dinner throughout the study. Two tubes (6 mL/tube) fasting venous blood sample were collected at the end of each phase, one sample into EDTA tubes for plasma and second set for serum. The blood tubes were centrifuged at 1200 x g for 10 minutes, followed by careful separation of plasma sample into three aliquots. One aliquot (2.0 mL) was immediately processed for total mRNAs and miRNAs purification [1].

The extraction of total mRNAs and miRNAs was carried out of randomly selected 11 samples of placebo and treatment groups of pre-treatments (at the start of the study), and post-treatment (at the end) from plasma of EDTA treated fresh whole blood by using total RNA purification kit # 17200 (NORGEN, Bioteck Corporation, Thorold, ON, Canada) and miRNA extraction, by using "Plasma/serum Circulation Purification Mini Kit (Slurry Format), Product # 51000 (NORGEN)" at the end of post-treatment. The purified total mRNAs and miRNAs were further purified and concentrated to 10.0 µL by using by Gene Jet RNA Clean up and Concentration Micro Kit (Thermo Scientific, EU, Lithuania). The concentrations of mRNA and miRNA were quantified by NanoDrop ND-1000 Spectrophotometer (NanoDrop, Wilmington, Delaware, USA) and purity of total mRNAs and miRNAs, were estimated by the ratios of 260/280 (2.02 - 2.08) of all samples. Purified total mRNAs and miRNAs were stored at -80 0C freezer or in the RNA STABLE TUBES (Biomaterica, 5627 Oberlin Derive, Suite 120, San Diego, CA. 92121) for further analyses. Purity of these RNAs was further determined in Genwize by their own instruments for quality control before putting into NG sequencing of mRNA and miRNA analyses were carried out (using Human's Transcriptome 2.0 or Human U133 plus 2.0; and miRNA 4.0) at Genwize, Suzhou 215123, China.

Statistical analyses:

Analysis of covariance (ANOVA) and Mann–Whitney U test was used to compare means of pre-treatment versus post-treatment. During validation phase Pearson correlation coefficient was applied to determine the correlation of miRNAs in both groups. Data reported as mean ± SD (Standard Deviation) in Tables. The statistical significance level was set at 5% (***P*** < 0.05).

Results

Effect of NS-3 treatment of people with T2DM on their gene expression carried out by IPA analyses of mRNAs and miRNAs.

The mRNA- and miRNA-sequence analyses was based on normalized FPKM > 2-fold change of 4000 gene ratios of post-treatment over pre-treatment of a mixture of NS-3 to people with T2DM. The data was uploaded into Ingenuity Pathway Analyses (IPA) for core analyses of different genes of direct and indirect relationship between focused molecules based on experimentally observed data and human databases in the Ingenuity Knowledge Base System (Ingenuity System, Redwood City, CA). The various genes of biological functions associated with diabetes were molecular functions, upstream regulators analysis, disease-based functions, and canonical pathways.

Molecular functions of miRNA

The IPA of molecular functions of miRNAs indicated fold change in gene expression of mainly 266 genes associated with diabetes mellitus out of 1373, covering several genes of various biomarkers. Out of these, 20 genes were up-regulated with log ratios of 10.4 – 2.0 (miR-29b-3p, miR-548h-5p, miR-624-5p, miR-361-5p, miR-130a-3p based on symbol) with significantly higher concentration. The other minor important miR-153-3p, miR-133a-3p, miR-153-3p and miR-95-3p (based on symbol) were shown in **Table 1A.** There were 27 down-regulated genes associated with diabetes **(Table 1B)**. The most important ones with significant log ratio of -9.1 - 4.3 were (based on symbol) miR-324-3p, miR-576-3p, miR-374c-5p, miR-4326, miR-5481, miR-4646-3p, 320b, miR-33-5p, miR-190a-5p, miR-7a-5p, miR-197-3p and miR-9-5p **(Table 1B)**.

Table 1A: IPA analysis (miRNA) of gene expression of "molecular functions" (up-regulated [20]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Genes ID	Expression Log Ratio	[a,b]Symbol
1	hsa-miR-29c-3p	10.4	miR-29b-3p (and other miRNAs w/seed AGCACCA)
2	hsa-miR-548ad-5p	8.1	miR-548h-5p (and other miRNAs w/seed AAAGUAA)
3	hsa-miR-624-5p	7.6	miR-624-5p (miRNAs w/seed AGUACCA)
4	hsa-miR-361-5p	7.5	miR-361-5p (miRNAs w/seed UAUCAGA)
5	hsa-miR-301a-3p	6.0	miR-130a-3p (and other miRNAs w/seed AGUGCAA)
6	hsa-miR-3912-3p	5.5	miR-3912-3p (miRNAs w/seed AACGCAU)
7	hsa-miR-1976	4.7	miR-1976 (and other miRNAs w/seed CUCCUGC)
8	hsa-miR-11401	4.0	miR-11401 (miRNAs w/seed CACGUCU)
9	hsa-miR-1284	4.0	miR-1284 (and other miRNAs w/seed CUAUACA)
10	hsa-miR-3605-3p	3.3	miR-3605-3p (miRNAs w/seed CUCCGUG)
11	hsa-miR-23c	2.0	miR-23a-3p (and other miRNAs w/seed UCACAUU)
12	hsa-miR-329-3p	1.6	miR-329-3p (and other miRNAs w/seed ACACACC)
13	hsa-miR-195-5p	1.4	miR-16-5p (and other miRNAs w/seed AGCAGCA)
14	hsa-miR-133a-3p	1.0	miR-133a-3p (and other miRNAs w/seed UUGGUCC)
15	hsa-miR-136-3p	1.0	miR-136-3p (miRNAs w/seed AUCAUCG)
16	hsa-miR-153-3p	1.0	miR-153-3p (miRNAs w/seed UGCAUAG)
17	hsa-miR-543	1.0	miR-543-3p (and other miRNAs w/seed AACAUUC)
18	hsa-miR-544b	1.0	miR-544b (miRNAs w/seed CCUGAGG)
19	hsa-miR-548av-3p	1.0	miR-548av-3p (miRNAs w/seed AAACUGC)
20	hsa-miR-95-3p	1.0	miR-95-3p (miRNAs w/seed UCAACGG)

Table 1B: IPA analysis (miRNA) of gene expression of "molecular functions" (down-regulated [27]) after NS-3 treated RNAs of people with T2DM

#	Genes ID	Expr Log Ratio	[a,b]Symbol
21	hsa-miR-324-3p	-9.1	miR-324-3p (miRNAs w/seed CCACUGC)
22	hsa-miR-576-3p	-8.0	miR-576-3p (miRNAs w/seed AGAUGUG)
23	hsa-miR-374c-5p	-7.8	miR-374c-5p (and other miRNAs w/seed UAAUACA)
24	hsa-miR-4326	-5.9	miR-4326 (miRNAs w/seed GUUCCUC)
25	hsa-miR-548l	-5.6	miR-548l (miRNAs w/seed AAAGUAU)
26	hsa-miR-4646-3p	-4.8	miR-4646-3p (miRNAs w/seed UUGUCCC)
27	hsa-miR-1292-5p	-4.6	miR-1247-3p (and other miRNAs w/seed GGGAACG)
28	hsa-miR-548aq-3p	-4.6	miR-548ae-3p (and other miRNAs w/seed AAAAACU)
29	hsa-miR-5695	-4.5	miR-5695 (miRNAs w/seed CUCCAAG)
30	hsa-miR-874-3p	-4.3	miR-874-3p (miRNAs w/seed UGCCCUG)
31	hsa-miR-320d	-2.6	miR-320b (and other miRNAs w/seed AAAGCUG)
32	hsa-miR-33b-5p	-2.6	miR-33-5p (and other miRNAs w/seed UGCAUUG)
33	hsa-miR-326	-1.6	miR-330-5p (and other miRNAs w/seed CUCUGGG)
34	hsa-miR-636	-1.4	miR-636 (miRNAs w/seed GUGCUUG)
35	hsa-miR-744-5p	-1.4	miR-744-5p (and other miRNAs w/seed GCGGGGC)
36	hsa-miR-589-5p	-1.3	miR-589-5p (and other miRNAs w/seed GAGAACC)
37	hsa-miR-618	-1.3	miR-618 (and other miRNAs w/seed AACUCUA)
38	hsa-miR-324-5p	-1.2	miR-324-5p (miRNAs w/seed GCAUCCC)
39	hsa-miR-190b-5p	-1.2	miR-190a-5p (and other miRNAs w/seed GAUAUGU)
40	hsa-miR-7-5p	-1.2	miR-7a-5p (and other miRNAs w/seed GGAAGAC)
41	hsa-miR-223-3p	-1.1	miR-223-3p (miRNAs w/seed GUCAGUU)
42	hsa-miR-501-3p	-1.1	miR-501-3p (and other miRNAs w/seed AUGCACC)
43	hsa-miR-197-3p	-1.0	miR-197-3p (and other miRNAs w/seed UCACCAC)
44	hsa-miR-487a-3p	-1.0	miR-154-3p (and other miRNAs w/seed AUCAUAC)
45	hsa-miR-526b-3p	-1.0	miR-17-5p (and other miRNAs w/seed AAAGUGC)
46	hsa-miR-184	-1.0	miR-184 (and other miRNAs w/seed GGACGGA)
47	hsa-miR-9-5p	-1.0	miR-9-5p (and other miRNAs w/seed CUUUGGU)

266 Analysis ready miRNA; Up >2 (95); Down >2 (171);
[a]Location = cytoplasm; [b]Types = mature micro RNA

Summary of IPA of up-regulated/down-regulated miRNAs of molecular functions

The results of miRNAs are summarized in **Table 2**. The data was divided into eight categories and
each category has mainly five functions (total 39). Out of 39 functions, only few are further investigated. The important functions among them are organism injury and abnormalities, inflammatory diseases, inflammatory response, cellular development, cellular growth and development, cell cycle, cell death survival, hepatic system development function, cardiac proliferation, cardiac infarction, liver inflammation, liver cirrhosis, gene expression and connective tissue disorder **(Table 2).** The most important miRNAs (10 of each), up-regulated are (miRNAs-29b-3p, miR-548h-5p, miR-624-5p, miR-361-5p, miR-130a-3p, miR-3912-3p, miR-1976, miR-11401, miR-1284, miR-3605-3p) and down-regulated (miR-324-3p, miR-576-3p, miR-374c-5p, miR-4326, miR-5481, miR-4646-3p, miR-548ae-3p, miR-1247-3p, miR-5695, miR-874-3p) associated with T2DM **(Table 2).**

The gene expression of first ten up-regulated and down-regulated of mRNA, miRNA, and paired mRNA-miRNA of molecular functions of each category are further discussed. All the information described in these sections are based on from the Google Search.

Summary of IPA of up-regulated miRNAs of molecular functions

The up-regulation of miR-29b-3p is an important modulator of glucose transporter member 4 (GLUT4) and hexokinase-2 (HK-2) in insulin resistance in diabetes and involves in the glycemic homeostasis impairment in diabetes **(Table 2).** It has an important protective role in cardiac fibrosis and is involved in various phases of wound healing. MicroR-548h-5p predicts urinary complications in humans, induces glucose-dependent insulin gene expression, and protects β-cells against TNF-α inhibition of insulin transcription and secretion, which could be used to detect early heart failure. MicroR-624-5p is directly involved with β-catenin and is an early biomarker of diabetic nephropathy in people with T2DM, and also involves in various tumor progression. It is one of the major risk factors in T2DM and plays an important role in pancreatic β-cells in response to metabolic, genetic, and inflammatory stress **(Table 2).**

MicroR-361-5p has a key role in vascular endothelial growth factor A (VEGFA), chronic inflammatory skin diseases and several types of skin cancer. Its plasma level plays an important role in late onset of hypogonadism and positively associated with serum testosterone in T2DM, metabolic syndrome, obesity, and cancer. It is a novel biomarker for late-onset of hypogonadism and causes reduction in the level of dipeptidyl peptidase-4. It suppresses VEGF expression and endothelial progenitor cell (EPC) activities. MicroR-130a-3p plays a central role in mitochondrial glucose metabolism in insulin secretion and sensitivity in T2DM, in nonalcoholic fatty liver disease, and is a potential circulating biomarker of T2DM. It is well established that impaired insulin secretion is a major factor in T2DM. The overexpression of miR-130a-3p modulates high glucose-induced MPC5 podocyte, and dysfunction through inhibition of TNF-α signaling. It has a major role in hepatic insulin sensitivity, liver steatosis, and controls the extracellular vesicles in T2DM, which are caused by metformin treatment **(Table 2).**

MicroR-3912-3p plays an important role in lipid metabolism and inflammation in T2DM and is also involved in molecular mechanism of diabetic cardiomyopathy, and miR-1976 is involved in the development of obesity and related diseases and implicated in the regulation of TP53 and CD40 and their relationship in response to specific weight-loss by diets in people with T2DM. It modulates endothelial cell function and regulates their secretion in T2DM **(Table 2).** MicroR-11401 is involved in several important diseases of T2DM, such as regulation of glucose stimulated insulin secretion and modulation of the cellular transcriptome at the post-transcriptional level. MicroR-1284 is in insulin resistance in people with T2DM, and miR-3605 is involved in inflammatory processes

contributing to the development of early T2DM such as non-alcoholic steatohepatitis (NASH) and inflammation of the visceral adipose tissue **(Table 2).**

Summary of IPA of down-regulated miRNAs of molecular functions

The down regulation of miR-324-3p is involved with hemolysis in people with T2DM. It would be a very good biomarker to detect onset of diabetes in future. It is also associated with vascular injury, and osteoporosis in people with T2DM. MicroRNA-576-3p plays important roles in several diseases of T2DM particularly in specific viruses, and miRNA374c-5p is involved in insulin sensitivity and resistance in T2DM **(Table 2).** It is also a good biomarker of diabetes. Its serum level indicates glycemic status and correlates with target mRNAs in pathways and modulates inflammatory process in obesity in people with T2DM. MicroR-4326 alters the bone matrix and reducing bone quality by impacting the vasculature in T2DM. It regulates the level of HbA1c in T2DM and is also involved in β-cell function and glycated hemoglobin, and its serum level is used to determine glucose tolerance in healthy people versus with T2DM **(Table 2).**

MicroR-5481 inhibits the expression of interleukin-1-receptor antagonist in pancreatic islets ofT2DM, leading to impaired insulin secretion, and decreased cell proliferation, and miR-4646-3p was modulated hypomagnesemia in T2DM. The deficiency of magnesium plays a role in the development of endothelial dysfunction and alters insulin function. The serum level of miR-548ae-3p regulates digestion, sexual function, and urination. It is also involved in metabolic syndrome and related conditions in people with T2DM **(Table 2).** It has a role in epigenetic modifications in hyperhomocysteinemia in T2DM. MicroR-1247-3p is involved in diabetic nephropathy, and miR-5695 has a role in hyperglycemia-induced oxidative stress in people with diabetes related to cardiovascular diseases. MicroR-874-3p induces chemotherapeutic agents and modulates β-cell function and insulin therapy. It might be a very good future biomarker for diagnosis of diabetes states **(Table 2).**

Table 2: Summary of IPA of various molecular functions (39) After NS-3 treated RNAs of people with type 2 diabetes mellitus.

	Name	*p* -Values	# Molecules
	Diseases and Disorders:		
1	Organismal Injury and Abnormalities	4.96E-02 - 2.08E-30	74
2	Reproductive System Disease	4.77E-02 - 2.08E-29	47
3	Inflammatory Disease	4.04E-02 - 5.29E-29	35
4	Inflammatory Response	3.38E-02 - 5.29E-29	33
5	Renal and Urological Disease	1.12E-04 - 5.29E-29	26
	Molecular and Cellular Functions:		
6	Cellular Development	4.70E-02 - 4.53E-07	29
7	Cellular Growth and Proliferation	4.70E-02 - 4.53E-07	28
8	Cellular Movement	3.38E-02 - 4.27E05	21
9	Cell Cycle	4.55E-02 - 1.18E-03	7
10	Cell Death and Survival	4.30E-02 - 1.21E-03	12
	Physiological System Development and Function:		
11	Digestive System Development and Function	3.77E-08 - 3.77E-08	6
12	Hepatic System Development and Function	3.77E-08 - 3.77E-08	6
13	Organ Development	4.55E-02 - 3.77E-08	9
14	Tissue Morphology	3.38E-02 - 8.03E004	6
15	Hematological System Development and Function	4.70E-02 - 1.03E-03	10
	Cardiotoxicity:		
16	Cardiac Fibrosis	1.49E-03 - 1.49E-03	5
17	Cardiac Dilation	1.61E-02 - 1.61E-02	5
18	Cardiac Enlargement	3.13E-01 - 1.61E-02	7
19	Cardiac Proliferation	4.55E-02 - 4.55E-02	2
20	Cardiac Infarction	9.33E-02 - 8.15E-02	3
	Hepatotoxicity:		
21	Liver Damage	3.77E-08 - 3.77E-08	6
22	Liver Inflammation/Hepatitis	3.77E-08 - 3.77E-09	6
23	Hepatocellular carcinoma	9.02e-05 - 9.02e-05	14
24	Liver Hyperplasia/Hyperproliferation	1.00E-00 - 9.02E-05	18
25	Liver Cirrhosis	1.88E-04 - 1.88-04	6
	Nephrotoxicity:		
26	Glomerular Injury	3.13E-01 - 5.29E-29	21
27	Renal Inflammation	5.29E-29 - 5.29E-29	20
28	Renal Nephritis	5.29E-29 - 5.29E-30	20
29	Renal Fibrosis	3.13E-01 - 3.13E-01	1
30	Renal Necrosis/Cell Death	1.00E-00 - 1.11E-00	1
	Top Toxicity Lists:	***p* -Values**	**Overlap**
31	Renal Ischemia-Reperfusion Injury	3.21E-04	25 % 2/8
32	MicroRNA Biomarker Panel (Mouse)		
33	Decreases Transmembrane Potential of	3.59E-01	0.8 % 1/129
34	Mitochondria and Mitochondrial MembraneTop of the Lists		
	Top Associated Network:		**Functions Score**
35	1. Organismal Injury & Abnormalities, Reproductive System disease, Glomerular Injury		39
36	2. Organismal Injury & abnormalities, reproductive System Disease, Glomerular Injury		30
37	3. Glomerular Injury, Inflammatory Disease, Inflammatory Response		28
38	4. Gene expression, Cancer, Connective Tissue Disorders		20
39	5. Cancer, Gastrointestinal Disease, Organiemal Injury & Abnormalities		18
	Gene Expression of Log Ratio of miRNAs of molecular functions:		
40	**Up-regulated:** miRNA-29b-3p, miRNA-548h-5p, miRNA-624-5p, miRNA-361-5p, miRNA-130a-3p, miRNA-3912-3p, miRNA-1976, miRNA-11401, miRNA-1284, miRNA-3605-3p.		
41	**Down-regulated:** miRNA-324-3p, miRNA-576-3pp, miRNA-374-5p, miRNA-4326, miRNA-5581, miRNA-4646-3p, miRNA-548ae-3p, miRNA-1247-3p, miRNA-5695, miRNA-874-3p.		

These results are supported by their heat map of miRNAs of pre-treatment vs post-treatment **(Figure 2).** Several miRNAs which are up-regulated in pre-treatment, were down-regulated significantly (miR-548aq-3p, 1292-5p, 83, 54, 50, 71, 35, 63, 25, 30, 77, 33, 48, 576-3p) after post-treatment. Whereas miR-3912-3p, 548au-5p, and 59, 301a-3p showed an opposite effect after post-treatment **(Figure 2).**

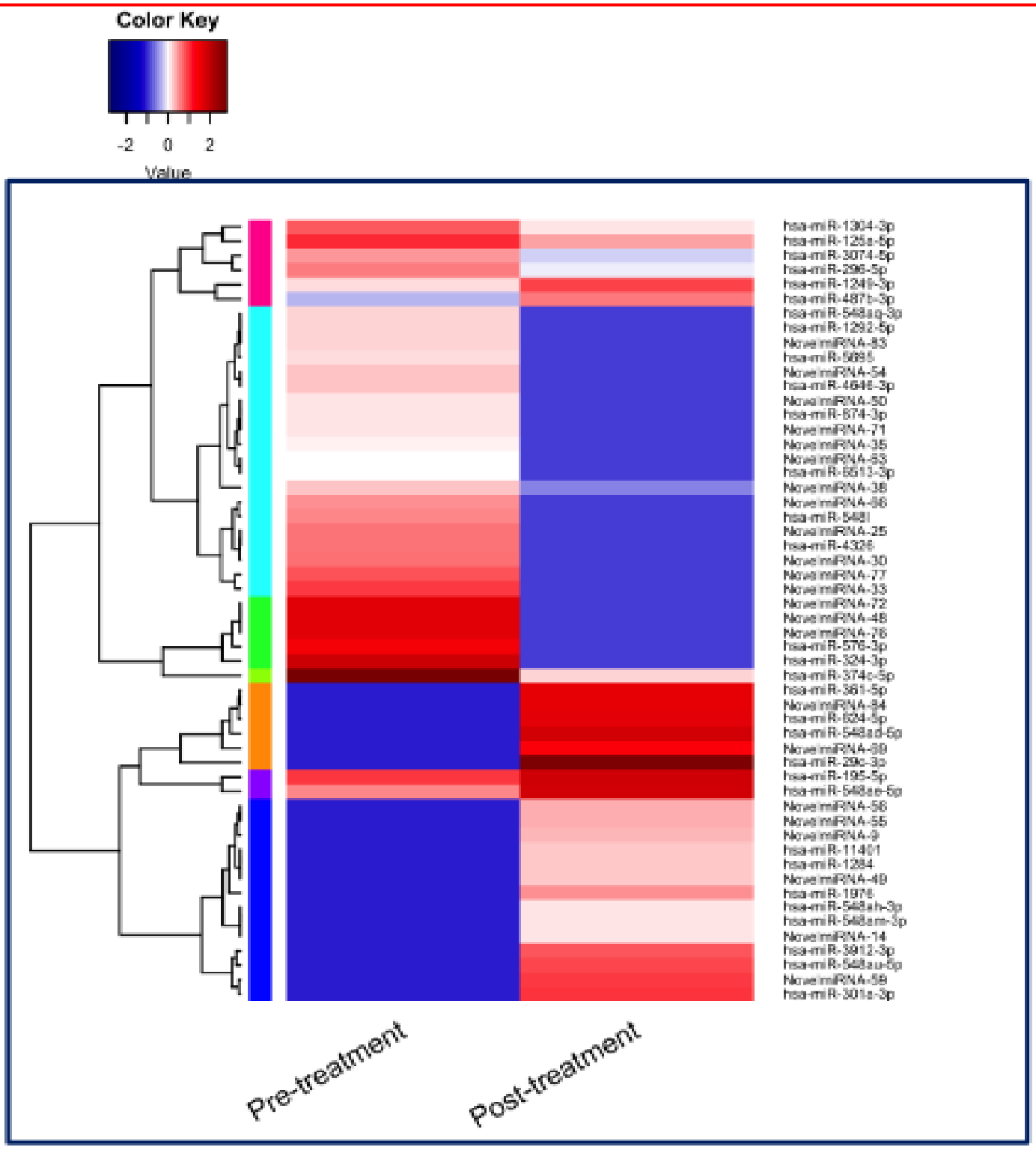


Figure 2: Effect of total RNAs of a mixture of NS-3 treated people with T2DM on heat map.

All the above genes expression results are also described by gene oncology (GO), Kyto Encyclopedia of genes and genomes (KEGG) and mRNA, miRNA, and paired mRNA-miRNA databases of pre-treatment vs post-treatment groups. In the category of GO system **(Figure 3),** all the genes of miRNAs are classified into three functional groups (biological-process, cellular-component, and molecular-function). Various genes are involved in binding, catalytic activity, signal transducer activity, transporter activity, molecular function regulator is notably represented in molecular-function category. Whereas, cell particle, organelle, organelle particle, membrane particle, macromolecular complex is in the cellular-component category. Molecular-function category indicates significant cellular process, single-organism metabolic process and development process **(Figure 3).**

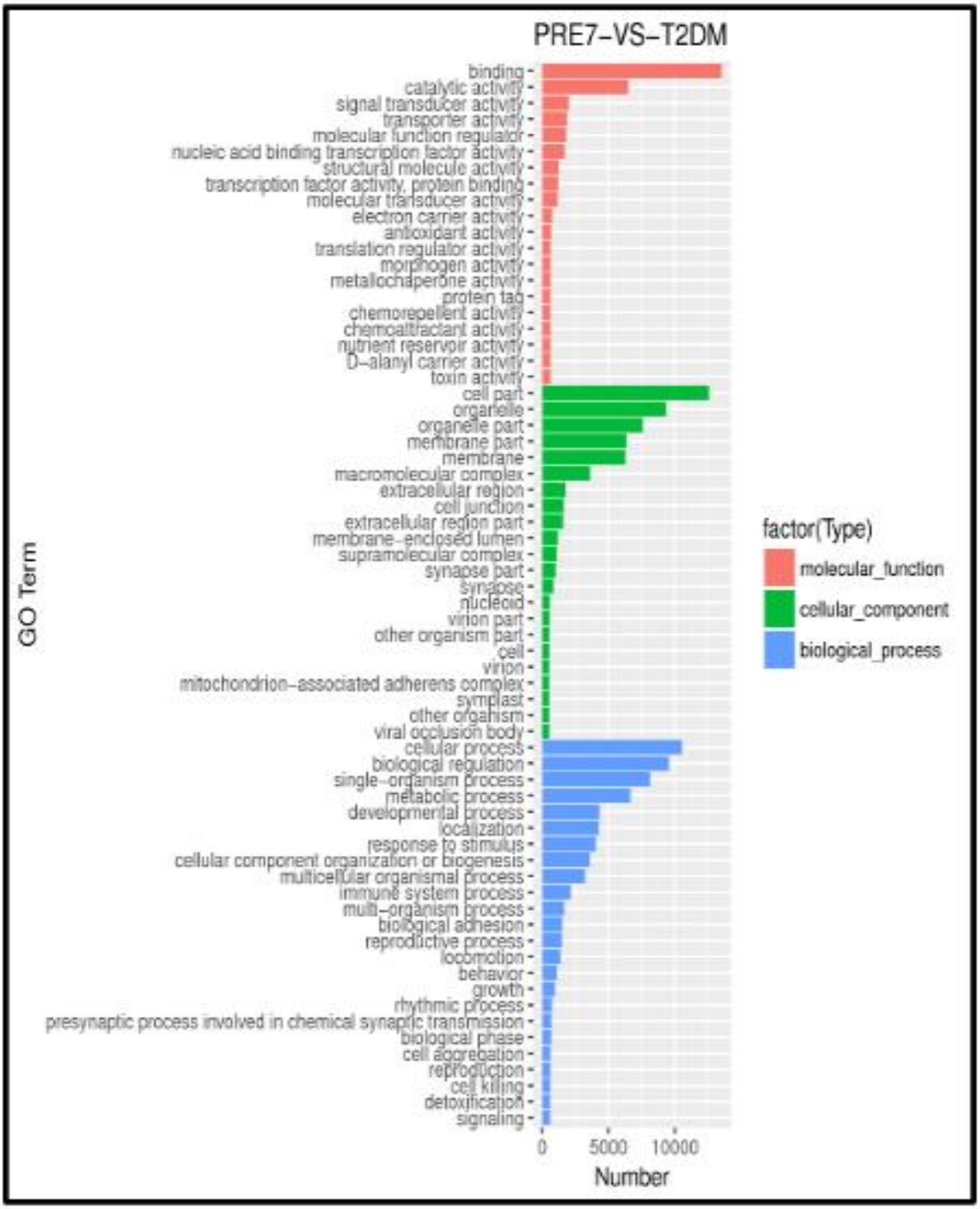


Figure 3: Effect of a mixture of NS-3 treated people with T2DM on Gene Oncology (GO) of miRNAs.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) of miRNAs is classified into thirty (30) biological pathways. Among them, most dominant pathways are RNA degradation, pathways in cancer, osteoclast differentiation, MAPK signaling pathways, insulin signaling pathway, Hippo signaling pathway, focal adhesion, dopaminergic synapsis, calcium signaling pathway and adipocytokine signaling pathway **(Figure 4).** There were eleven (11) pathways out of thirty (30), which are changed significantly as observed by KEGG analysis after NS-3 post-treatment group of people with T2DM **(Figure 4).**

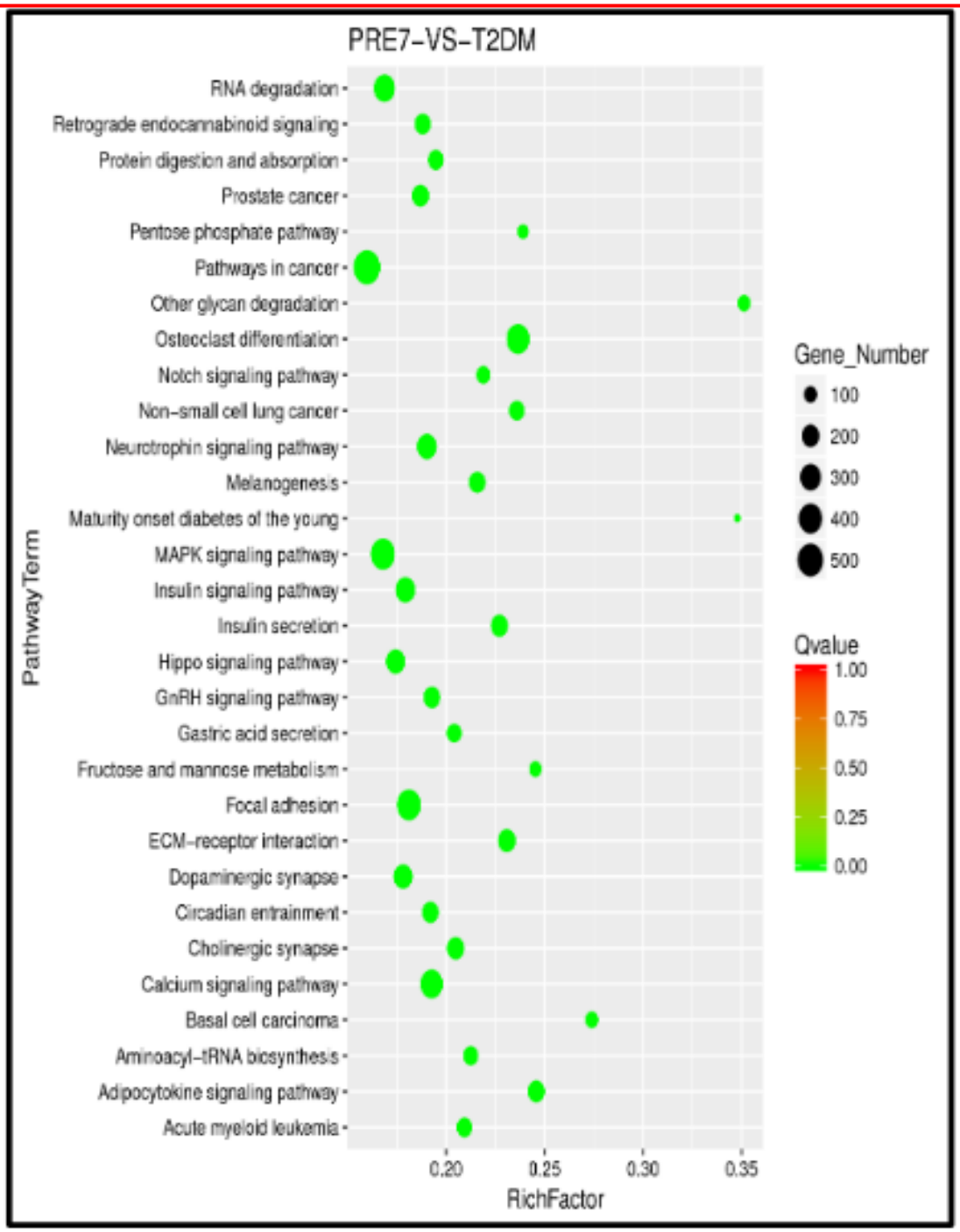


Figure 4: Effect of a mixture of NS-3 treated people with T2DM on scatter plot of various plot of Various pathways in Kyoto Encyclopedia of genes and genomes (KEGG) of miRNAs.

Biological Functions of messengerRNA (mRNA) of people with T2DM.

The IPA analyses of mRNAs of post-treatment over pre-treatment obtained from people with T2DM after administration of a mixture of NS-3 for 24-weeks are grouped under six sections (molecular functions, up-stream regulators, diseases functions, network functions, ingenuity canonical pathways, and summary of the IPA analyses). The molecules functions describe fold changes in gene expression of up-regulated (1666) and down-regulated (88), a total of 1754 genes. Out of these, 42 (up-regulated) with log ratios of 14.8 – 1.0 and 17 (down-regulated) with log ratios of -22.2 - -1.1 are the most important fold change in gene expression associated with diabetes **(Tables 3A & 3B).** The log ratios of gene expression, gene functions, location of each gene are clearly described in these **Tables.** The summary of above describes various functions (61) of mRNAs has been provided in **Table 4A.** The functions of various genes associated with diabetes are discussed below (**Table 4A;** section 62-up-regulated and section 63-down-regulated).

Table 3A: IPA analysis of gene expression of mRNAs of "molecular functions" (up-regulated [42]) after NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	ID	Symbol	Expr Log Ratio	Entrez Gene Name	Location	Type(s)
1	ENSG00000275215	RNA5-8SN3	14.8	RNA, 5.8S ribosomal N3	Other	other
2	ENSG00000201183	RNVU1-3	14.5	RNA, variant U1 small nuclear 3	Other	other
3	ENSG00000241069	CTD_3141N221	12.6	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
4	ENSG00000234648	AL1621513	12.3	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
5	ENSG00000273711	RP5_1021I208	11.9	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
6	ENSG00000241588	RN7SL484P	10.6	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
7	ENSG00000279337	CTD_2349P217	10.4	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
8	ENSG00000203326	ZNF525	10.2	zinc finger protein 525	Nucleus	transcription regulator
9	ENSG00000198538	ZNF28	10.0	zinc finger protein 28	Nucleus	transcription regulator
10	ENSG00000211716	TRBV9	10.0	T cell receptor beta variable 9	Plasma Membrane	other
11	ENSG00000235576	LINC01871	9.8	long intergenic non-protein coding RNA 1871	Other	other
12	ENSG00000276185	TP53TG1_2	9.7	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
13	ENSG00000282939	TRBV7-2	9.7	T cell receptor beta variable 7-2	Other	other
14	ENSG00000269981	RP11_34P1316	9.6	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
15	ENSG00000242616	GNG10	9.5	G protein subunit gamma 10	Plasma Membrane	other
16	ENSG00000227191	TRGC2	8.9	T cell receptor gamma constant 2	Other	other
17	ENSG00000239951	IGKV3-20	8.6	immunoglobulin kappa variable 3-20	Extracellular Space	other
18	ENSG00000065518	NDUFB4	8.5	NADH:ubiquinone oxidoreductase subunit B4	Cytoplasm	transporter
19	ENSG00000211801	TRAV21	8.2	T cell receptor alpha variable 21	Other	other
20	ENSG00000148484	RSU1	4.6	Ras suppressor protein 1	Cytoplasm	other
21	ENSG00000141232	TOB1	4.1	transducer of ERBB2, 1	Nucleus	transcription regulator
22	ENSG00000170989	S1PR1	4.0	sphingosine-1-phosphate receptor 1	Plasma Membrane	G-protein coupled receptor
23	ENSG00000060971	ACAA1	3.9	acetyl-CoA acyltransferase 1	Cytoplasm	enzyme
24	ENSG00000110324	IL10RA	3.6	interleukin 10 receptor subunit alpha	Plasma Membrane	transmembrane receptor
25	ENSG00000134539	KLRD1	2.8	killer cell lectin like receptor D1	Plasma Membrane	transmembrane receptor
26	ENSG00000170458	CD14	2.8	CD14 molecule	Plasma Membrane	transmembrane receptor
27	ENSG00000172349	IL16	2.5	interleukin 16	Extracellular Space	cytokine
28	ENSG00000063046	EIF4B	2.1	eukaryotic translation initiation factor 4B	Cytoplasm	translation regulator
29	ENSG00000150045	KLRF1	2.1	killer cell lectin like receptor F1	Plasma Membrane	transmembrane receptor
30	ENSG00000160211	G6PD	2.1	glucose-6-phosphate dehydrogenase	Cytoplasm	enzyme
31	ENSG00000136888	ATP6V1G1	2.1	ATPase H+ transporting V1 subunit G1	Cytoplasm	transporter
32	ENSG00000145779	TNFAIP8	2.1	TNF alpha induced protein 8	Cytoplasm	other
33	ENSG00000159128	IFNGR2	1.9	interferon gamma receptor 2	Plasma Membrane	transmembrane receptor
34	ENSG00000027697	IFNGR1	1.9	interferon gamma receptor 1	Plasma Membrane	transmembrane receptor
35	ENSG00000077238	IL4R	1.9	interleukin 4 receptor	Plasma Membrane	transmembrane receptor
36	ENSG00000185201	IFITM2	1.9	interferon induced transmembrane protein 2	Cytoplasm	other
37	ENSG00000110801	PSMD9	1.7	proteasome 26S subunit, non-ATPase 9	Cytoplasm	transcription regulator
38	ENSG00000014216	CAPN1	1.3	calpain 1	Cytoplasm	peptidase
39	ENSG00000099341	PSMD8	1.2	proteasome 26S subunit, non-ATPase 8	Cytoplasm	other
40	ENSG00000110955	ATP5F1B	1.0	ATP synthase F1 subunit beta	Cytoplasm	transporter
41	ENSG00000149925	ALDOA	1.0	aldolase, fructose-bisphosphate A	Cytoplasm	enzyme
42	ENSG00000105122	RASAL3	1.0	RAS protein activator like 3	Cytoplasm	other

Table 3B: IPA analysis of gene expression of mRNAs of "molecular functions" (down-regulated [17]) after NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	ID	Symbol	Expr Log Ratio	Entrez Gene Name	Location	Type(s)
43	ENSG00000244734	HBB	-22.2	hemoglobin subunit beta	Cytoplasm	transporter
44	ENSG00000269246	CTC_246B1810	-13.1	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
45	ENSG00000229122	AGBL5-IT1	-8.6	AGBL5 intronic transcript 1	Other	other
46	ENSG00000244232	RN7SL698P	-8.5	RNA, 7SL, cytoplasmic 698, pseudogene	Other	other
47	ENSG00000226024	COX5BP7	-8.4	cytochrome c oxidase subunit 5B pseudogene 7	Other	other
48	ENSG00000262624	RP11_104H159	-7.9	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
49	ENSG00000242861	RP11_285F72	-7.7	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
50	ENSG00000163993	S100P	-4.9	S100 calcium binding protein P	Cytoplasm	other
51	ENSG00000198887	SMC5	-4.4	structural maintenance of chromosomes 5	Nucleus	other
52	ENSG00000225195	RP11_338E212	-3.4	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
53	ENSG00000260482	CTD_2196E149	-3.3	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
54	ENSG00000275527	CTD_3154N52	-2.7	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
55	ENSG00000134697	GNL2	-1.5	G protein nucleolar 2	Nucleus	enzyme
56	ENSG00000233461	RP11_295G202	-1.4	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other
57	ENSG00000128829	EIF2AK4	-1.3	eukaryotic translation initiation factor 2 alpha kinase	Cytoplasm	kinase
58	ENSG00000103342	GSPT1	-1.1	G1 to S phase transition 1	Cytoplasm	translation regulator
59	ENSG00000267681	CTD_3199J236	-1.1	chondroitin sulfate proteoglycan 4 pseudogene 3 Y	Other	other

Summary of IPA of up-regulated mRNAs of molecular functions

The up-regulated gene expression of mRNAs genes (RNA5-8SN3, RNVU1-3, CTD-3141N221, AL1621513, RP5-10211208, RP7SL484P, CTD-2349P217, ZNF525, ZNF28 and TRBV9) collectively are involved in insulin secretion, insulin resistance, inflammation, infectious diseases, immunological diseases, and cellular functions **(Table 3A).** RNA5-8SN3 is the copy of 5.8 ribosomal RNA on chromosome 21 and has an important role in immunity by regulating the expression of a large number, of biologically active compounds of diabetes. The up-regulation of RNVU1-3 is resulted in the development of T2DM, and CTD-3141N221 controls the level of intracellular, magnesium, which regulated insulin-mediated glucose-uptake and vascular function. AL1621513 is involved with oxidative stress, which induces complications of diabetes, such as neuropathy, retinopathy, stroke **(Table 3A),** and RP5-10211208 plays an important role in nervous system. ZNF525 and ZNF28 genes are involved in the transcriptional regulation, and TRBV9 is involved in T cell beta variable coding gene [9].

Summary of IPA of down-regulated mRNAs of molecular functions

The down-regulated gene expression of mRNAs genes (HBB, CTC-246B1810, AGBL5IT1, RN7SL698P, COX5BP7, RP11-104H159, RP11-285F72, S100P, SMC5, and R *R11-338E212*) collectively are involved in β -cell, glycemic control, energy metabolism, inflammation, and regulation of proliferation **(Table 3B).** HBB functions as a theranostic

molecule and acts also as a hemoglobin glycation in people with T2DM, and CTC-246B1810 is involved cytokines and β-cell biology in T2DM. Whereas, AGBL5IT1 is associated with ATP/GTP binding protein, also used as a CRISPR-clones for T2DM, and RN7SL698P is associated with role of inflammatory cytokines and its complications in diabetes. COX5BP7 modulates proper glycemic control in diabetes **(Table 3B).**

RP11-104H159 modulates the function of IL-1 by inhibiting the β-cell function and by destroying β-cell mass, and RP11-285F72 has effect on chronic kidney disease in people with T2DM. Whereas, S100P is involved in the regulation of proliferation, differentiation, apoptosis, CA2+ homoeosatasis, energy metabolism, inflammation, and migration/invasion through interactions with several target proteins including receptors and transcription factors **(Table 3B).** SMC5 is involved in β-cell functions, and R *RR11-338E212* with depression observed in people with T2DM. These results are further supported by summary of various genomic functions of mRNA of pre-treatment vs post-treatment **(Figures 5A, 5B)** of people with T2DM. The values of various genes of pre- vs post-dose are summarized in **Table 4B.** All the genes are up-regulated two-ford to three-fold after NS-3 treatment of people of T2DM, except exonic; splicing down-regulated to 0.0, which shows significant effectiveness of NS-3 treatment in diabetes **(Table 4B).**

Table 4A: Summary of IPA analysis of various functions (61) of NS-3 treated mRNAs of people with type 2 diabetes mellitus.

#	Name	*p* -Values	Overlap
	Top Canonical Pathways:		
1	EIF2 Signaling	6.81E-36	36.0 % 81/221
2	Mitochondrial Dysfunction	4.49E-27	35 61/171.7%
3	Oxidative Phosphorylation	2.29E-28	44.0 % 48/109
4	Sirtuin Signaling Pathway	3.51E-20	24.3 % 71/292
5	TOR Signaling	3.72E-20	28.0 % 59/211
	Top Upstream Regulators:		**Predicted Activation**
6	CD 437	9.28E-40	Inhibited
7	5-fluorouracil	2.80E-38	Inhibited
8	RICTOR	6.59E-38	Inhibited
9	ST1926	8.49E+33	Inhibited
10	IL15	1.53E-32	Activated
	Diseases and Disorders:		**# Molecules**
11	Inflammatory Response	2.95E-09 - 4.67E-82	633
12	nfectious Diseases	1.91E-09 - 3.71E-66	447
13	Immunological Disease	4.24E-09 - 3.47E-42	600
14	Connective Tissue Disorders	8.76E-10 - 1.52E-32	308
15	Inflammatory Disease	2.95E-09 - 1.52E-32	417
	Molecular and Cellular Functions:		
16	Cellular Compromise	3.55E-09 - 3.19E-81	265
17	Protein Synthesis	7.21E-10 - 4.09E46	397
18	Cell Death and Survival	4.07E-09 - 3.69E-43	701
19	Cellular Development	4.62E-10 - 1.94E-38	512
20	Cellular Growth and Proliferation	6.86E-10 - 1.94E-38	532
	Physiological System Development and Function:		
21	Hematological System Development and Function	3.30E-09 - 1.94E-38	467
22	Lymphoid Tissue Structure and Development	2.57E-09 - 1.94E-38	337
23	Immune Cell Trafficking	3.30E09 - 9.64E-37	306
24	Tissue Morphology	3.08E-09 - 4.14E-27	338
25	Organ Morphology	1.05E-09 - 2.13E-19	132
	Assays: Clinical Chemistry and Hematology:		
26	Increased Levels of Hematocrit	2.01E-04 - 2.01E-04	18
27	Increased Levels of Creatinine	2.67E-01 - 1.13E-02	9
28	Increased Levels of Blood Urea Nitrogen	2.67E-01 - 1.13E-02	4
29	Decreased Levels of Albumin	5.15E-02 - 5.15E-02	5
30	Increased Levels of Albumin	6.22E-01 - 6.41E-02	3
	Cardiotoxicity:		
31	Cardiac Inflammation	4.28E-01 - 2.70E-05	26
32	Cardiac Dilation	5.61E-01 - 4.00E-05	43
33	Cardiac Enlargement	1.00E-00 - 4.00E-05	78
34	Cardiac Necrosis/Cell Death	2.59E-01 - 6.74E-05	40
35	Cardiac Damage	1.99E-01 - 7.63E-03	16
	Hepatotoxicity:		
36	Hepatocellular carcinoma	5.93E-01 - 6.12E-08	106
37	Liver Hyperplasia/Hyperproliferation	1.00E-00 - 6.12E008	531
38	Liver Inflammation/Hepatitis	6.22E-01 - 7.66E-06	60
39	Liver Damage	6.22E-01 - 2.11E-05	52
40	Liver Fibrosis	3.93E-01 - 1.31E-04	28
	Nephrotoxicity:		
41	Renal Necrosis/Cell Death	4.50E-01 - 4.07E-09	82
42	Renal Proliferation	3.62E-01 - 9.06E-05	41
43	Glomerular Injury	5.61E-01 - 2.55E-04	68
44	Renal Damage	5.61E-01 - 6.00E-43	43
45	Renal Inflammation	5.61E-01 - 1.49E-03	53
	Regulators:	**Disease & Functions**	**Consistency Score**
46	**1.** sirolimus	Cell death of osteosarcoma cells	4.69
47	**2.** RICTOR	Cell death of osteosarcoma cells	4.36
48	**3.** MYCN	Cell death of osteosarcoma cells	4.13
49	**4.** MYC	MYC Cell death of osteosarcoma cells	3.88
50	**5.** ATF4	Organismal death	3.87
	Top Toxicology List:	*p* -Values	Overlap
51	Mitochondrial Dysfunction	6.49E-27	35.5 % 61/172
52	Renal Necrosis/Cell Death	1.25E-09	14.4 % 82/569
53	NRF2-mediated Oxidative Stress Response	2.47E-05	14.9 % 36/241
54	Increases Liver Damage	7.34E-05	19.2 % 19/99
55	Decreases Transmembrane Potential of		
56	Mitochondria and Mitochondrial Membrane Top Lists of Pathway	1.31E+04	17.1 % 22/129
	Top Network:		
	Associated Network		**Function Score**
57	1. Developmental Disorder, Heredity disorder, Metabolic diseases.		42
58	2. Infectious Diseases, Small Molecular Biochemistry, Cellular Assembly & Organization		40
59	3. RNA Post-Transcriptional Modification, Cellular Signaling, Post-Translation Modification		40
60	4. RNA Damage & Repair, Protein Synthesis, Cancer		40
61	5. Protein synthesis, RNA Post-Transcriptional Modification, Heredity Disorder		40
	Gene Expression of Log Ratios of mRNA of molecular functions.		
62	**Up-regulated: RNA5-8SN3, RNVU1-3, CTD-3141N221, ZNF525, ZNF28, TRBV9, LINCO1871, TP53TG1-2, TRBV7-2, RP11-34P1316.**		
63	**Down-regulated: HBB, CTC-246B1810, AGBL-IT1, RN7SL698P, COX5BP7, RP11-104H159, RP11-285F72, S100P, SMC5, RP11-338E212.**		

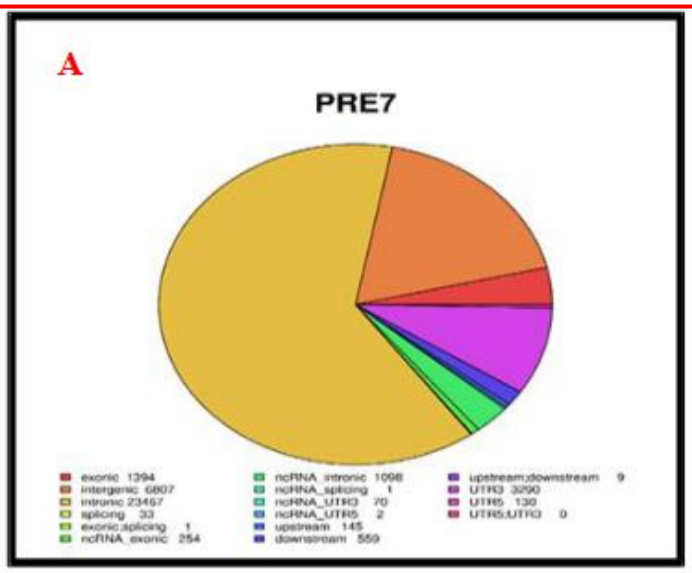


Figure 5A: Pre-dose.

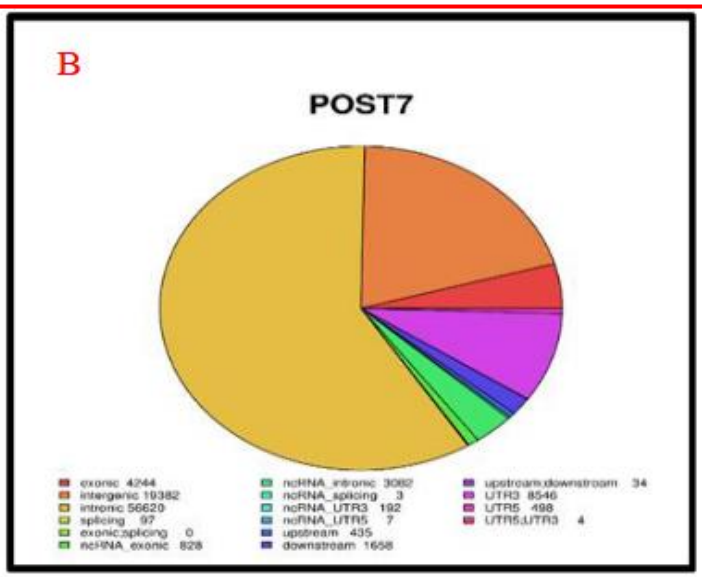


Figure 5B: Post-dose.

Table 4B: Summary of various genomic functions of NS-3 treated mRNAs of pre-dose versus post-dose of people with type 2 diabetes mellitus.

#	Gene ID	Pre-dose values*	Post-dose values*
1	Exonic	1394	4244
2	Intergenic	6807	19382
3	Interogenic	23467	56620
4	Splicing	33	97
5	Exonic; Splicing	1	0
6	ncRNA; Exonic	254	828
7	ncRNA; Intronic	1098	3082
8	ncRNA; Splicing	1	3
9	ncRNA; UTR3	70	192
10	ncRNA; UTR5	2	7
11	Upstream	145	435
12	Downstream	559	1658
13	Upstream; Downstream	9	34
14	UTR3	3290	8546
15	UTR5	130	498
16	UTR3; UTR5	0	4

*Data generated from Figure 5A & 5B: mRNAs Functions.

The category of gene oncology (GO) histogram for mRNAs are also classified into three functional groups (biological-process, cellular-component, and molecular-function). The genes involve significantly in molecular-function category are binding, catalytic activity, molecular transducer activity, transporter activity, enzyme regulator activity, and molecular function regulator **(Figure 6).** Whereas, cell particle, organelle particle, membrane particle, macromolecular complex represented in the cellular-component category. The molecular function category indicates significant cellular process, biological regulation, and single-organism metabolic process in response to stimulus and immune system processes **(Figure 6).**

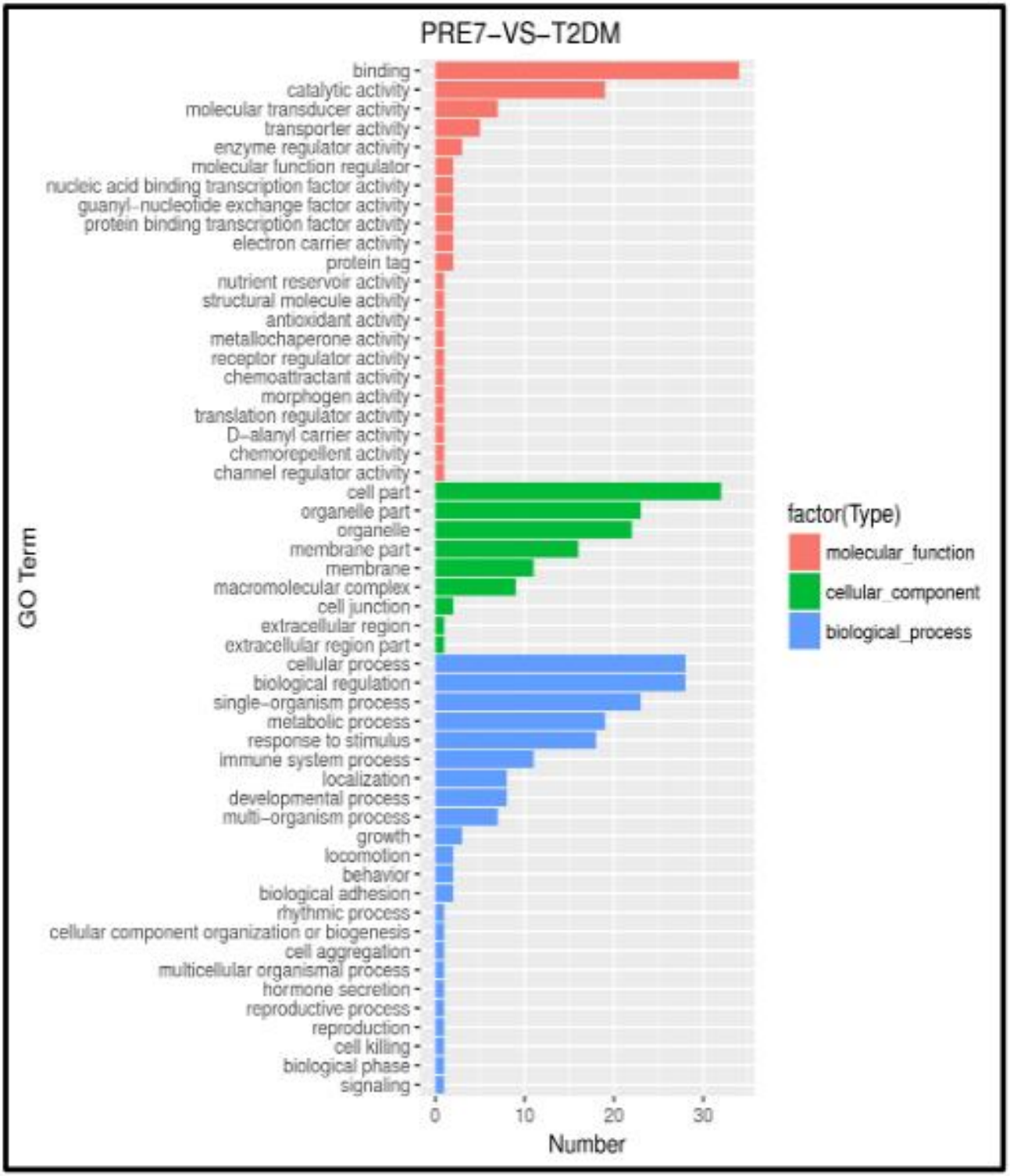


Figure 6: Effect of a mixture of NS-3 treated people with T2DM on Gene Oncology (GO) of mRNAs.

Kyoto Encyclopedia of Genes and Genomes (KEGG) is also classified into thirty (30), biological pathways. Among them, most dominant pathways are toll-like receptor signaling pathway, Parkinson's disease, oxidative phosphorylation, NOD-like receptor signaling pathway, NF-*k*B signaling pathway, Huntington's disease, herpes simplex infection, hepatitis C, hepatitis B, Epstein-Barr virus infection, cytosolic DNA-sensing pathway, cytokine-cytokine receptor interaction, basal transcription factors and AMPK signaling pathway **(Figure 7).** Eleven (11) pathways out of thirty (30) are changed significantly as observed by KEGG analysis after post-treatment group of people with T2DM **(Figure 7).**

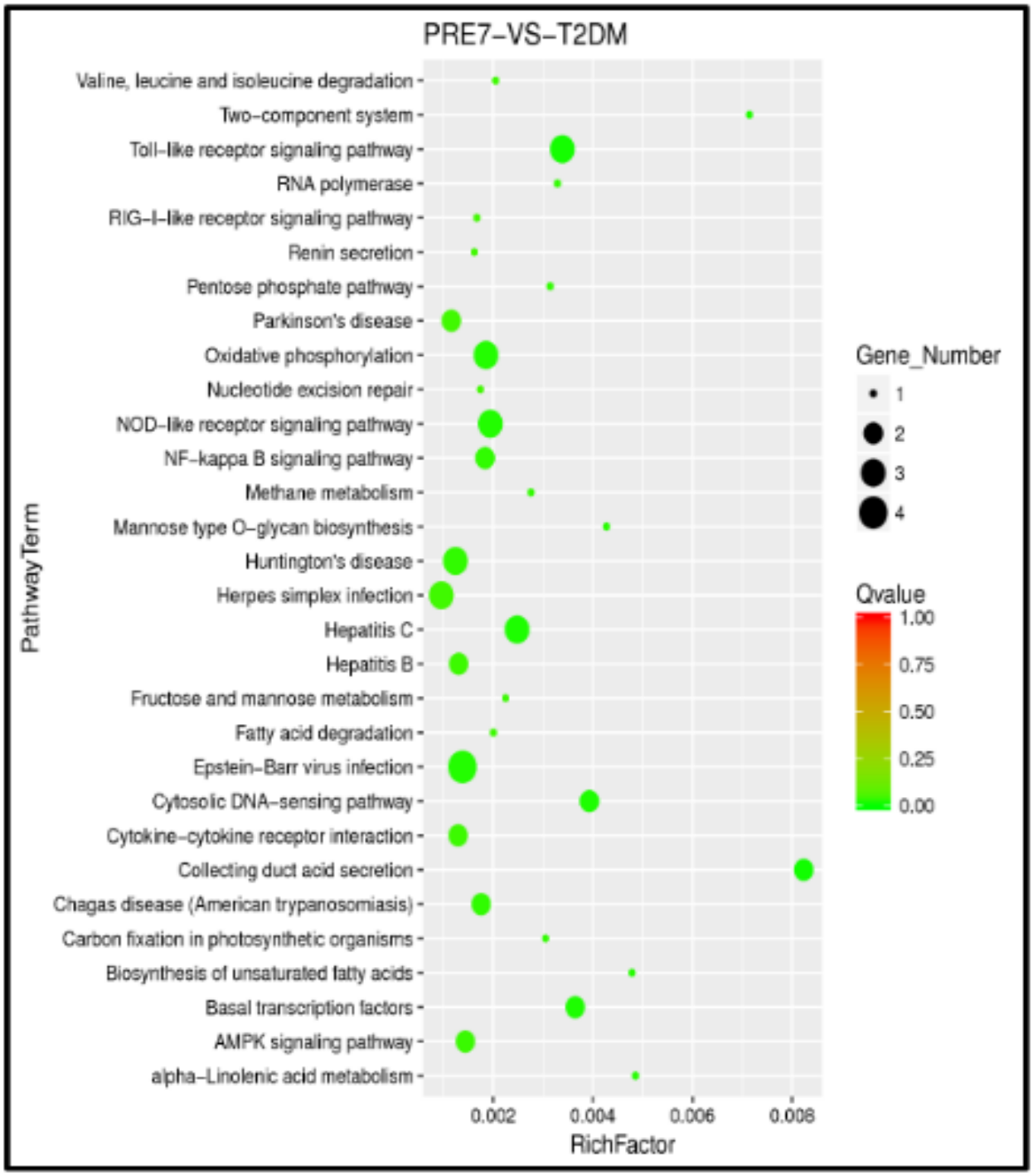


Figure 7: Effect of a mixture of NS-3 treated people with T2DM on scatter plot of various pathways in Kyoto encyclopedia of genes and genomes (KEGG) of mRNAs.

Interaction of biological functions of paired mRNAs-miRNAs of people with T2DM.

The interaction of IPA analyses of paired mRNAs-miRNAs of post-treatment over pre-treatment obtained from people with T2DM after administration of a mixture of NS-3 for 24-weeks are also described under same six sections (molecular functions, up-stream regulators, diseases functions, network functions, ingenuity canonical pathways, and summary of the IPA analyses) as reported for mRNAs. The molecules functions of paired mRNAs-miRNA are found fold changes in gene expression of up-regulated (38) with log ratios of 10.2 – 1.0 and down-regulated (4) with log ratios of -1.1 – 1.3 **(Tables 5A, 5B)** out of a total 1000 genes. The summary of paired mRNAs-miRNAs IPA analyses is described in 54 categories associated with diabetes **(Table 6).** The functions of first ten genes are up-regulated (ZNF525, ZNF28, GNG10, NDUFB4, ORMDL1, S100B, BCKDHA, OXA1L, SBF1, RSU1) and four down-regulated (SET, RAB31, BRD4, KANK2) of paired mRNAs-miRNAs of molecular functions will be further discussed **(Table 6).**

Table 5A: IPA analysis of paired (mRNA-miRNA) gene expression of "molecular functions" (up-regulated [38]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Gene ID	Expression Log Ratio	Symbol	Entrez Gene Name	Location	Type(s)
1	ENSG00000203326	10.2	ZNF525	zinc finger protein 525	Nucleus	transcription regulator
2	ENSG00000198538	10.0	ZNF28	zinc finger protein 28	Nucleus	transcription regulator
3	ENSG00000242616	9.5	GNG10	G protein subunit gamma 10	Plasma Membrane	other
4	ENSG00000065518	8.5	NDUFB4	NADH:ubiquinone oxidoreductase subunit B4	Cytoplasm	transporter
5	ENSG00000128699	8.5	ORMDL1	ORMDL sphingolipid biosynthesis regulator 1	Cytoplasm	other
6	ENSG00000160307	8.3	S100B	S100 calcium binding protein B	Cytoplasm	other
7	ENSG00000248098	7.9	BCKDHA	branched chain keto acid dehydrogenase E1, alpha pol	Cytoplasm	enzyme
8	ENSG00000155463	4.7	OXA1L	OXA1L mitochondrial inner membrane protein	Cytoplasm	enzyme
9	ENSG00000100241	4.6	SBF1	SET binding factor 1	Plasma Membrane	phosphatase
10	ENSG00000148484	4.6	RSU1	Ras suppressor protein 1	Cytoplasm	other
11	ENSG00000066322	4.5	ELOVL1	ELOVL fatty acid elongase 1	Cytoplasm	enzyme
12	ENSG00000114125	4.5	RNF7	ring finger protein 7	Nucleus	enzyme
13	ENSG00000113328	4.4	CCNG1	cyclin G1	Nucleus	other
14	ENSG00000154473	4.4	BUB3	BUB3 mitotic checkpoint protein	Nucleus	other
15	ENSG00000103254	4.2	FAM173A	family with sequence similarity 173 member A	Other	other
16	ENSG00000144895	4.2	EIF2A	eukaryotic translation initiation factor 2A	Cytoplasm	translation regulator
17	ENSG00000153563	4.1	CD8A	CD8a molecule	Plasma Membrane	other
18	ENSG00000100796	4.0	PPP4R3A	protein phosphatase 4 regulatory subunit 3A	Plasma Membrane	other
19	ENSG00000110324	3.6	IL10RA	interleukin 10 receptor subunit alpha	Plasma Membrane	transmembrane receptor
20	ENSG00000185627	2.6	PSMD13	proteasome 26S subunit, non-ATPase 13	Cytoplasm	peptidase
21	ENSG00000172349	2.5	IL16	interleukin 16	Extracellular Space	cytokine
22	ENSG00000161921	2.5	CXCL16	C-X-C motif chemokine ligand 16	Extracellular Space	cytokine
23	ENSG00000275302	2.2	CCL4	C-C motif chemokine ligand 4	Extracellular Space	cytokine
24	ENSG00000128272	2.1	ATF4	activating transcription factor 4	Nucleus	transcription regulator
25	ENSG00000120129	2.0	DUSP1	dual specificity phosphatase 1	Nucleus	phosphatase
26	ENSG00000077238	1.9	IL4R	interleukin 4 receptor	Plasma Membrane	transmembrane receptor
27	ENSG00000070831	1.5	CDC42	cell division cycle 42	Cytoplasm	enzyme
28	ENSG00000204389	1.5	HSPA1A/HSPA1B	heat shock protein family A (Hsp70) member 1A	Cytoplasm	enzyme
29	ENSG00000125818	1.5	PSMF1	proteasome inhibitor subunit 1	Cytoplasm	other
30	ENSG00000010278	1.4	CD9	CD9 molecule	Plasma Membrane	other
31	ENSG00000139318	1.3	DUSP6	dual specificity phosphatase 6	Cytoplasm	phosphatase
32	ENSG00000086061	1.3	DNAJA1	heat shock protein family (Hsp40) member A1	Nucleus	other
33	ENSG00000014216	1.3	CAPN1	calpain 1	Cytoplasm	peptidase
34	ENSG00000131143	1.2	COX4I1	cytochrome c oxidase subunit 4I1	Cytoplasm	enzyme
35	ENSG00000163636	1.1	PSMD6	proteasome 26S subunit, non-ATPase 6	Cytoplasm	enzyme
36	ENSG00000130741	1.1	EIF2S3	eukaryotic translation initiation factor 2 subunit gamma	Cytoplasm	translation regulator
37	ENSG00000126353	1.0	CCR7	C-C motif chemokine receptor 7	Plasma Membrane	G-protein coupled receptor
38	ENSG00000168685	1.0	IL7R	interleukin 7 receptor	Plasma Membrane	transmembrane receptor

Table 5B: IPA analysis ofpaired (mRNA-miRNA) gene expression of "molecular functions" (down-regulated [4]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Gene ID	Expression Log Ratio	Symbol	Entrez Gene Name	Location	Type(s)
39	ENSG00000119335	-1.1	SET	SET nuclear proto-oncogene	Nucleus	phosphatase
40	ENSG00000168461	-1.1	RAB31	RAB31, member RAS oncogene family	Cytoplasm	enzyme
41	ENSG00000141867	-1.1	BRD4	bromodomain containing 4	Nucleus	kinase
42	ENSG00000197256	-1.3	KANK2	KN motif and ankyrin repeat domains 2	Nucleus	transcription regulator

#	Table 6: IPA analysis 0f paired (mRNA-miRNA) gene expression of "various functions" after NS-3 treated RNAs of people with type 2 diabetes mellitus.		
	Top Canonical Pathways:	***p*-Values**	**Overlap**
1	Integrin Signaling	3.58E-17	17.2 % 37/215
2	Actin Nucleation by ARP-WASP Complex	6.28E-13	26.4 % 19/729
3	Phospholipase C Signaling	9.09E-12	12.8% 33/257
4	Regulation of Actin-based Motility by Rho	1.19E-11	21.3 % 20/94
5	Cdc42 Signaling	1.94E-11	15.6 % 26/167
	Top Upstream Regulators:		**Predicted Activation**
6	IL15	1.85E-18	Activated
7	CD 437	1.12E-16	Inhibited
8	CD3	1.16-E15	Activated
9	TP53	1.48E-15	Activated
	Diseases and Disorders:		**# Molecules**
10	Inflammatory Response	1.27E-06 - 8.51E-46	294
11	Infectious Diseases	9.18E-06 - 2.15E-27	197
12	Immunological Disease	9.74E-07 - 1.55E-19	257
13	Inflammatory Disease	5.74E-08 - 8.00E-18	188
	Molecular and Cellular Functions:		
14	Cellular Compromise	1.13E-06 - 8.51E-46	132
15	Cell Death and Survival	1.27E-06 - 1.71E-26	321
16	Cellular Movement	1.27E-06 - 1.73E-23	228
17	Cellular Development	1.28E-06 - 4.02E-22	260
18	Cellular Growth and Proliferation	1.28E-06 - 4.04E-22	265
	Physiological System Development and Function:		
19	Immune Cell Trafficking	1.27E-06 - 6.79E-23	152
20	Hematological System Develop & Function	1.28E-06 - 2.68E-22	230
21	Tissue Morphology	9.48E-07 - 9.66E-18	166
22	Organ Morphology	2.63E-07 - 2.38E-14	70
	Clinical Chemistry and Hematology:		
23	Increased Levels of Hematocrit	1.26E-02 - 1.26E-02	8
24	Increased Levels of Creatinine	3.63E-01 - 2.40E-02	5
25	Increased Levels of ALT	2.27E-01 - 9.19E-02	3
26	Decreased Levels of Albumin	1.76e-01 - 9.83E-02	3
	Cardiotoxicity:		
27	Cardiac Necrosis/Cell Death	1.21E-01 - 3.99E-06	25
28	Cardiac Enlargement	1.00E-00 - 8.21E-06	50
29	Cardiac Dysfunction	5.09E-01 - 8.76E-05	15
30	Cardiac Inflammation	4.65E-01 - 1.54E-04	13
31	Cardiac Fibrosis	5.53E-01 - 2.90E-02	20
	Hepatotoxicity:		
32	Liver Necrosis/Cell Death	1.00E00 - 8.77E06	25
33	Liver Damage	5.33E-01 - 2.96E-05	29
34	Liver Proliferation	2.53E-01 - 3.17E-05	20
35	Liver Inflammation/Hepatitis	5.33E-01 - 4.34E-04	29
36	Hepatocellular carcinoma	4.40E-01 - 315E-03	54
	Nephrotoxicity:		
37	Renal Necrosis/Cell Death	1.64E-01 - 1.34E-08	48
38	Renal Proliferation	2.02E-01 - 3.58E-06	27
39	Renal Inflammation	1.00E-00 - 8.21E-04	29
40	Renal Nephritis	1.00E-00 - 8.21E-05	29
	Top Regulator Effect on Networks:	**Disease & Functions**	**Consistency Score**
41	1 PI3K (complex)	Cell viability of tumor cell lines,	3.317
42	2 poly rI:rC-RNA	Migration of mononuclear leukocytes,	3.317
43	3 BCR (complex)	Migration of cells,	3.175
44	4 IFNG	Recruitement of granulocytes.	3.175
	Top Toxicology List:	***p*-Values**	**Overlap**
45	Renal Necrosis/Cell Death	8.08E-10	8.4 % 18/569
46	Mitochondrial Dysfunction	1.33E-07	12.2 % 21/172
47	Cardiac Necrosis/Cell Death	1.24E-05	8.3 % 25/301
48	Liver Necrosis/Cell Death	3.48E-05	7.8 % 25/320
49	Cardiac Fibrosis	5.35E-05	8.6 % 20/232
	Top Network		
	Associated Network Functions		**Score**
50	1. Infectious Diseases, Cell Death & Survival, RNA Post-transcriptional Modification		46
51	2. Cellular Movement, Cellular Assembly & Organization, Cell morphology		44
52	3. Cellular Compromise, Inflammatory Response, Cellular Development		42
53	4. Gene Expression, RNA Demage & Repair, RNA Post-transcriptional Modification		42
54	5. RNA Demage & Repair, Protein synthesis, Cancer		42
	Gene Expression of Log Ratio of paired miRNA-mRNA of molecular functions		
55	**Up-regulated: ZNF525, ZNF28, GNG10, NDUFB4, ORMDL1, S100B, BCKDHA, OXA1L, SBF1, RSU1.**		
56	**Down-regulated: KANK2, BRD4, RAB31, SET.**		

Summary of IPA of up-regulated of paired mRNA-miRNAs of molecular functions

The biological function of up-regulated ZNF525 is involved in regulation of transcription in diabetes, and ZNF28 has a function as transcriptional repressor, and mutations in this gene resulted in transient neonatal diabetes. Whereas NDUFB4 plays an important role of glucotoxicity in β -cell differentiation, ORMDL1 modulates function of β-cells of the pancreas in people with T2DM, S100B implicates in cell proliferation, cytoskeletal dynamics, and tumorigenesis. BCKDHA is an inner mitochondrial enzyme complex of branched-chain alpha-keto acid (BCAA) dehydrogenase (BCKD) which catalyzed the second major step in the catabolism of the branched-chain amino acids leucine, isoleucine, and valine **(Table 5A).**

BCKD complex is composed of three catalytic components: a heterotetrameric (alpha2-beta2) branched-chain alpha-keto acid decarboxylase (E1), and OXA1L is required for the insertion of integral membrane proteins into the mitochondrial inner membrane, which is important for the activity and assembly of cytochrome oxidase. It is also required for the correct biogenesis of ATP synthase and complex I in mitochondria. SBE1 is involved in the degradation of Bcr-AB1 through ubiquitination via the lysosomal pathway. The RSU1 plays a role in Ras signal transduction, and also contributes to the regulation of cell adhesion and spreading by PINCH1-dependent and independent mechanisms **(Table 5A).**

Summary of IPA of down-regulated of paired mRNA-miRNAs of molecular functions.

The down-regulated SET is an encoded protein, inhibits acetylation of nucleosomes, especially histone H4, by histone acetylases in diabetes. RAB31 modulates vesicle trafficking, and over-overexpression of RAB31 inhibits insulin stimulated Glut4 translocation. **(Table 5B).** BRD4 is a histone acetylated reader protein, binds to acetylated lysine residues on histone and non-histone proteins to act as transcriptional regulators, resulting in the activation or repression of gene transcription. KANK2 gene is involved in making SRC interacting SIP protein which regulates steroid receptor coactivators (SRCs). SIP combined with SRCs in cytoplasm and prevents these proteins from entering the nucleus to activate genes, thus SIP is responsible to control the gene activity **(Table 5B).**

The present result collectively identified 92 mRNAs that are up-regulated with negative correlation of 14 miRNAs (miR-3074-5p, miR-5481, miR-125a-5p, miR-374c-3p, miR-548-3p, miR-576-3p, miR-1292-5p, miR-296-5p, miR-1304-3p, miR-374-3p, miR-4326, miR-6513-3p, miR-5695, miR-4646-3p), which are down-regulated of post-treatment group of T2DM **(Figure 8).** It is clear from this Figure 8 that a single miRNA can regulate multiple targets of mRNAs, for example miRNA-5481 targets several mRNAs associated with T2DM **(Figure 8).**

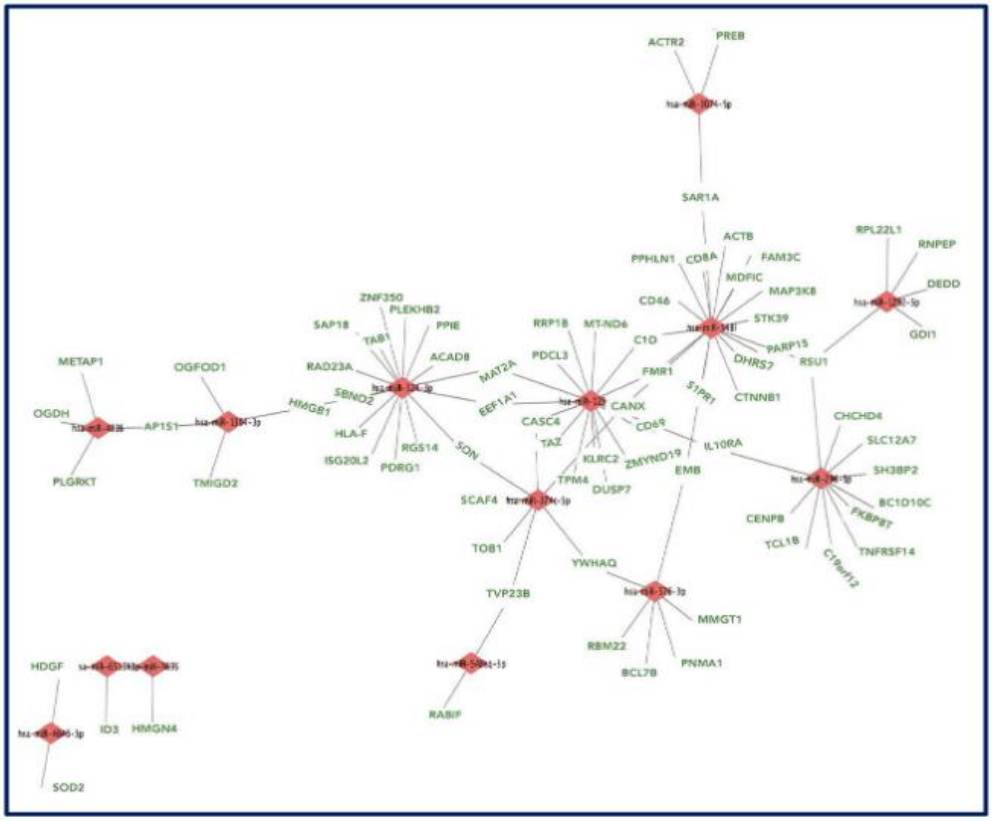


Figure 8: mRNAs up-regulated and mRNAs down-regulated.

The interaction network of miR-29b-3p and other miRNAs w/seed AGCACCA.

The interaction network of miR-29b-3p is generated using genes/molecules/pathways based on experimentally observed evidence of directly interacting with miR-29b-3p in people with T2DM. The molecules are organized according to their subcellular locations such as extracellular space, plasma membrane, cytoplasm, nucleus, or "other" category **(Figure 9)**. The transcriptome expression data of network indicates red shades denote intensities of up-regulation, whereas green shades denote intensities of down-regulation of genes, and gray denote no change in post-treatment group compared with pre-treatment group. For

example, LOXL2 enzyme coding gene has log2FC of -2.38 and has a darker green shade as compared to LAMC1 which has log2FC of only -0.19 and hence has lighter shade of green. Whereas the location of TUG1 is specified in "other" category **(Figure 9).** There were eighteen (18) red up-regulated genes (FAM3C, AGO2, PPM1D, FAM3C, SPARC, ANKHD1/ANKD1/EIF4, EBP3, TP53, MLF1, PURA, CNOT8, DNMT3A, PP1C, 2FP36L, HMGN3, MYBL2, TUBB2A, ZFP36L), four (4) green down-regulated genes (LOXL2, COL1A2, LAMC1, GPR37) and seven (7) gray no change genes (COL5A2, SHFOOM2, TRIM9, DCP2, RERE, NAV5, HDAC4).

In summary, all results of experimental design with respect to mRNA, miRNA, and paired mRNA-miRNA of IPA analyses data of gene expression profile of post-treatment has been described by Venn diagrams, incorporating network images and canonical pathways. The network images indicate 9 mRNA, 10 miRNA and overlap of 29 paired mRNA-miRNA **(Figure 10),** indicating their functions in **Table 7.** The most specific network images relevant to present study are from mRNA category (RNA-trafficking, cell mediated immune response, inflammatory disease, lipid metabolism) and from miRNA (immunological disease, immune-cell-trafficking, hematological-disease) as reported in **Table 7.** Similarly, Venn diagram of canonical pathways indicating 74 mRNAs, 23 miRNAs, and 174 paired mRNA-miRNA **(Figure 11),** and list of all the pathways are listed in **Table 8**. The list of these pathways of mRNAs, miRNAs and paired mRNA-miRNA have confirmed earlier above describe results.

Table 7: Summary of network based on "Venn Diagram" of IPA analysis of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#		Total
	A. Paired mRNA-miRNA	**29**
1	Cell_Death_and_Survival	
2	Cell-To-Cell_Signaling_and_Interaction	
3	Cellular_Compromise	
4	Gene_Expression	
5	RNA_Post-Transcriptional_Modification	
6	Cellular_Growth_and_Proliferation	
7	Infectious_Diseases	
8	Cell_Cycle	
9	RNA_Damage_and_Repair	
10	DNA_Replication	
11	Cellular_Function_and_Maintenance	
12	Cellular_Movement	
13	Cell_Signaling	
14	Cellular_Assembly_and_Organization	
15	Cellular_Development	
16	Post-Translational_Modification	
17	Protein_Synthesis	
18	Developmental_Disorder	
19	Hematological_System_Development_and_Function	
20	Metabolic_Disease	
21	Cancer	
22	Inflammatory_Response	
23	Recombination	
24	Cardiovascular_Disease	
25	Energy_Production	
26	Hereditary_Disorder	
27	Small_Molecule_Biochemistry	
28	and_Repair	
29	Embryonic_Development	
	B. mRNAs	**9**
1	Dermatological_Diseases_and_Conditions	
2	Organismal_Injury_and_Abnormalities	
3	Amino_Acid_Metabolism	
4	RNA_Trafficking	
5	Cell-mediated_Immune_Response	
6	Molecular_Transport	
7	Inflammatory_Disease	
8	Lipid_Metabolism	
9	Reproductive_System_Development_and_Function	
	C. miRNAs	**10**
1	Tissue_Morphology	
2	Immunological_Disease	
3	Nervous_System_Development_and_Function	
4	Nucleic_Acid_Metabolism	
5	Immune_Cell_Trafficking	
6	Cell_Morphology	
7	Connective_Tissue_Disorders	
8	Lymphoid_Tissue_Structure_and_Development	
9	Neurological_Disease	
10	Hematological_Disease	

Table 8: Summary of Caninocal Pathways based on "Venn Diagram" of IPA analysis of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	A. Paired mRNA-miRNA (Total-174)			#	B. mRNAs (Total 74)
1	Death_Receptor_Signaling	96	Dendritic_Cell_Maturation	1	Communication_betw_Innate_&_Adaptive_Imune_Cells
2	Lipid_Antigen_Presentation_by_CD1	97	Ephrin_Receptor_Signaling	2	FAT10_Signaling_Pathway
3	P2Y_Purigenic_Receptor_Signaling_Pathway	98	HER-2_Signaling_in_Breast_Cancer	3	Nucleotide_Excision_Repair_Pathway
4	OX40_Signaling_Pathway	99	Axonal_Guidance_Signaling	4	Eumelanin_Biosynthesis
5	Apelin_Endothelial_Signaling_Pathway	100	Sumoylation_Pathway	5	Tryptophan_Degradation_III_(Eukaryotic)
6	Actin_Nucleation_by_ARP-WASP_Complex	101	GP6_Signaling_Pathway	6	Endometrial_Cancer_Signaling
7	IL-7_Signaling_Pathway	102	PI3K/AKT_Signaling	7	CNTF_Signaling
8	Granzyme_B_Signaling	103	Renal_Cell_Carcinoma_Signaling	8	Melanocyte_Development_and_Pigmentation_Signaling
9	Protein_Ubiquitination_Pathway	104	Glucocorticoid_Receptor_Signaling	9	Glutaryl-CoA_Degradation
10	Phagosome_Maturation	105	Endocannabinoid_Developing_Neuron_Pathway	10	GDNF_Family_Ligand-Receptor_Interactions
11	Neuregulin_Signaling	106	Cdc42_Signaling	11	Fc_Epsilon_RI_Signaling
12	Role_of_Macrophages,_Fibroblasts_and_Endothelial_	107	PKC?_Signaling_in_T_Lymphocytes	12	Amyloid_Processing
13	Cells_in_Rheumatoid_Arthris"	108	Erythropoietin_Signaling	13	Spermidine_Biosynthesis_I
14	AK_Signaling	109	Granulocyte_Adhesion_and_Diapedesis	14	FLT3_Signaling_in_Hematopoietic_Progenitor_Cells
15	Tec_Kinase_Signaling	110	Regulation_of_IL-2_Expr_in_Activated_and_Anergic_T_	15	PPAR_Signaling
16	mTOR_Signaling	111	Cholecystokinin/Gastrin-mediated_Signaling	16	G-Protein_Coupled_Receptor_Signaling
17	Spermine_and_Spermidine_Degradation_I	112	14-3-3-mediated_Signaling	17	Adrenomedullin_signaling_pathway
18	Acute_Myeloid_Leukemia_Signaling	113	PI3K_Signaling_in_B_Lymphocytes	18	Thrombopoietin_Signaling
19	Phospholipase_C_Signaling	114	Unfolded_protein_response	19	Sertoli_Cell-Sertoli_Cell_Junction_Signaling
20	Chemokine_Signaling	115	?-Adrenergic_Signaling	20	ErbB4_Signaling
21	Regulation_of_eIF4_and_p70S6K_Signaling	116	Germ_Cell-Sertoli_Cell_Junction_Signaling	21	Oncostatin_M_Signaling
22	IL-4_Signaling	117	Synaptogenesis_Signaling_Pathway	22	ERK5_Signaling
23	Telomere_Extension_by_Telomerase	118	HMGB1_Signaling	23	VEGF_Family_Ligand-Receptor_Interactions
24	Telomerase_Signaling	119	Cardiac_Hypertrophy_Signaling	24	Non-Small_Cell_Lung_Cancer_Signaling
25	T_Cell_Receptor_Signaling	120	Opioid_Signaling_Pathway	25	NER_Pathway
26	"PD-1,_PD-L1_cancer_immunotherapy_pathway"	121	Antigen_Presentation_Pathway	26	G_Protein_Signaling_Mediated_by_Tubby
27	Dopamine_Receptor_Signaling	122	iNOS_Signaling	27	Gluconeogenesis_I
28	Calcium-induced_T_Lymphocyte_Apoptosis	123	BAG2_Signaling_Pathway	28	Leukotriene_Biosynthesis
29	Oxidative_Phosphorylation	124	Semaphorin_Signaling_in_Neurons	29	Endoplasmic_Reticulum_Stress_Pathway
30	Ephrin_B_Signaling	125	Granzyme_A_Signaling	30	NADH_Repair
31	D-myo-inositol-5-phosphate_Metabolism	126	IL-8_Signaling	31	Adenine_and_Adenosine_Salvage_I
32	Glioma_Signaling	127	Clathrin-mediated_Endocytosis_Signaling	32	Complement_System
33	RhoA_Signaling	128	Tight_Junction_Signaling	33	Mitotic_Roles_of_Polo-Like_Kinase
34	Systemic_Lupus_Erythematosus_In_T_Cell_Signaling_Pathways	129	"D-myo-inositol_(1,4,5,6)-Tetrakisphosphate_Biosynthes	34	G?s_Signaling
35	Molecular_Mechanisms_of_Cancer	130	Production_of_Nitric_Oxide_and_Reactive_Oxygen_Spe	35	Glutathione_Redox_Reactions_II
36	Type_I_Diabetes_Mellitus_Signaling	131	Regulation_of_Actin-based_Motility_by_Rho	36	JAK/Stat_Signaling
37	PTEN_Signaling	132	Integrin_Signaling	37	Relaxin_Signaling
38	Mitochondrial_Dysfunction	133	Epithelial_Adherens_Junction_Signaling	38	Estrogen_Receptor_Signaling
39	LK_Signaling	134	Role_of_NFAT_in_Cardiac_Hypertrophy	39	ErbB2-ErbB3_Signaling
40	CCR3_Signaling_in_Eosinophils	135	Nur77_Signaling_in_T_Lymphocytes	40	Fatty_Acid_?-oxidation_I
41	Actin_Cytoskeleton_Signaling	136	IGF-1_Signaling	41	Hereditary_Breast_Cancer_Signaling
42	IL-1_Signaling	137	Renin-Angiotensin_Signaling	42	Allograft_Rejection_Signaling
43	Cytotoxic_T_Lymphocyte-mediated_Apoptosis_of_Target_Cells	138	NF-kB_Activation_by_Viruses	43	IL-6_Signaling
44	Glioma_Invasiveness_Signaling	139	T_Helper_Cell_Differentiation	44	IL-2_Signaling
45	B_Cell_Receptor_Signaling	140	Androgen_Signaling	45	NF-kB_Signaling
46	G_Beta_Gamma_Signaling	141	Leukocyte_Extravasation_Signaling	46	Cleavage_and_Polyadenylation_of_Pre-mRNA
47	Hematopoiesis_from_Pluripotent_Stem_Cells	142	G?q_Signaling	47	Graft-versus-Host_Disease_Signaling

48	Endocannabinoid_Cancer_Inhibition_Pathway	143	CTLA4_Signaling_in_Cytotoxic_T_Lymphocytes	48	PFKFB4_Signaling_Pathway
49	Cyclins_and_Cell_Cycle_Regulation	144	Cardiac_Hypertrophy_Signaling_(Enhanced)	49	IL-15_Production
50	Cell_Cycle:_G1/S_Checkpoint_Regulation	145	CREB_Signaling_in_Neurons	50	Iron_homeostasis_signaling_pathway
51	Crosstalk_between_Dendritic_Cells_and_Natural_Killer_Cells	146	Prostate_Cancer_Signaling	51	eNOS_Signaling
52	3-phosphoinositide_Degradation_Prolactin_Signaling	147	3-phosphoinositide_Biosynthesis	52	IL-10_Signaling
53	Regulation_of_Cellular_Mechanics_by_Calpain_Protease	148	NRF2-mediated_Oxidative_Stress_Response	53	Neuroinflammation_Signaling_Pathway
54	IL-12_Signaling_and_Production_in_Macrophages	149	Agrin_Interactions_at_Neuromuscular_Junction	54	PEDF_Signaling
55	G?i_Signaling	150	Dopamine-DARPP32_Feedback_in_cAMP_Signaling	55	Prostanoid_Biosynthesis
56	Aldosterone_Signaling_in_Epithelial_Cells	151	Virus_Entry_via_Endocytic_Pathways	56	"Role_of_JAK1,_JAK2_&_TYK2_in_Interfe_Signaling"
57	Th1_Pathway	152	RhoGDI_Signaling	57	Myc_Mediated_Apoptosis_Signaling
58	Aryl_Hydrocarbon_Receptor_Signaling	153	Fc?_Receptor-mediated_Phagocytosis_in_	58	Ceramide_Signaling
59	Polyamine_Regulation_in_Colon_Cancer	154	Macrophages_and_Monocytes	59	Neurotrophin/TRK_Signaling
60	fMLP_Signaling_in_Neutrophils	155	HIPPO_signaling	60	UVC-Induced_MAPK_Signaling
61	Colorectal_Cancer_Metastasis_Signaling	156	IL-3_Signaling	61	Endothelin-1_Signaling
62	LPS-stimulated_MAPK_Signaling	157	Paxillin_Signaling	62	Sperm_Motility
63	Remodeling_of_Epithelial_Adherens_Junctions	158	VEGF_Signaling	63	GM-CSF_Signaling
64	RAR_Activation	159	GNRH_Signaling	64	Gap_Junction_Signaling
65	Role_of_NFAT_in_Regulation_of_the_Immune_Response	160	Thrombin_Signaling	65	Amyotrophic_Lateral_Sclerosis_Signaling
66	Signaling_by_Rho_Family_GTPases	161	Macropinocytosis_Signaling	66	Antiproliferative_Role_of_Somatostatin_Receptor_2
67	Breast_Cancer_Regulation_by_Stathmin1	162	Chronic_Myeloid_Leukemia_Signaling	67	PPAR?/RXR?_Activation
68	NGF_Signaling	163	Th1_and_Th2_Activation_Pathway	68	T_Cell_Exhaustion_Signaling_Pathway
69	PAK_Signaling	164	Sirtuin_Signaling_Pathway	69	SAPK/JNK_Signaling
70	p70S6K_Signaling	165	CCR5_Signaling_in_Macrophages	70	IL-15_Signaling
71	Induction_of_Apoptosis_by_HIV1	166	Reelin_Signaling_in_Neurons	71	ErbB_Signaling
72	HGF_Signaling	167	Rac_Signaling	72	Melanoma_Signaling
73	Huntington's_Disease_Signaling	168	Cardiac_?-adrenergic_Signaling	73	Autoimmune_Thyroid_Disease_Signaling
74	Insulin_Receptor_Signaling	169	Protein_Kinase_A_Signaling	74	G?12/13_Signaling
75	Th2_Pathway	170	Glioblastoma_Multiforme_Signaling	#	**C. miRNAs (Total-23)**
76	Tumoricidal_Function_of_Hepatic_Natural_Killer_Cells	171	Hypoxia_Signaling_in_the_Cardiovascular_System	1	TNFR1_Signaling
77	B_Cell_Development	172	Interferon_Signaling	2	Apelin_Cardiomyocyte_Signaling_Pathway
78	TGF-?_Signaling	173	DNA_Methylation_and_Transcriptional_Repression_Sign	3	Wnt/?-catenin_Signaling
79	Phagosome_Formation	174	Glycolysis_I	4	Osteoarthritis_Pathway
80	Melatonin_Signaling			5	p38_MAPK_Signaling
81	iCOS-iCOSL_Signaling_in_T_Helper_Cells			6	TWEAK_Signaling
82	Synaptic_Long_Term_Potentiation			7	Antiproliferative_Role_of_TOB_in_T_Cell_Signaling
83	Primary_Immunodeficiency_Signaling			8	Adipogenesis_pathway
84	"D-myo-inositol_(3,4,5,6)-tetrakisphosphate_Biosynthesis"			9	L-17A_Signaling_in_Fibroblasts
85	CXCR4_Signaling			10	cAMP-mediated_signaling
86	Agranulocyte_Adhesion_and_Diapedesis			11	Triacylglycerol_Biosynthesis
87	CDK5_Signaling			12	Pathogenesis_of_Multiple_Sclerosis
88	Superpathway_of_Inositol_Phosphate_Compounds			13	Hepatic_Fibrosis_/_Hepatic_Stellate_Cell_Activation
89	EIF2_Signaling			14	Atherosclerosis_Signaling
90	Caveolar-mediated_Endocytosis_Signaling			15	IL-17A_Signaling_in_Gastric_Cells
91	CD28_Signaling_in_T_Helper_Cells			16	CDP-diacylglycerol_Biosynthesis_I
92	Sphingosine-1-phosphate_Signaling			17	nNOS_Signaling_in_Neurons
93	ERK/MAPK_Signaling			18	Neuropathic_Pain_Signaling_In_Dorsal_Horn_Neurons
94	Apoptosis_Signaling			19	Role_of_PKR_in_Interferon_Induction_and_Antiviral_Response
95	Systemic_Lupus_Erythematosus_Signaling			20	Corticotropin_Releasing_Hormone_Signaling
				21	Sucrose_Degradation_V_(Mammalian)
				22	Phosphatidylglycerol_Biosynthesis_II_(Non-plastidic)
				23	Mechanisms_of_Viral_Exit_from_Host_Cells

The IPA analyses of mRNAs of NS-3 treatment of people with T2DM after 24-weeks, also reported data of up-stream regulators **(Tables 9A, 9B),** diseases function **(Tables 10A, 10B),** network functions **(Table 11)** and canonical pathways **(Table 12)** and for paired interaction of mRNAs-miRNAs up-stream regulators **(Tables 13A, 13B),** diseases function **(Tables 14A, 14B),** network functions **(Table15),** canonical signaling pathways **(Table 16)** in **Supplement File**.

Discussion

The present investigation describes the effect of NS-3 (natural products) administered to people with T2DM on gene expression of mRNA, miRNA, and their interaction of paired mRNA-miRNA by NG-sequencing **(Tables 1 - 6)**. The individual number with prediabetes and diabetes will increase to 482 million people in 2040. The current biomarkers, CRP, HbA1c, and HOMA-IR have limitations and are inaccurate under certain clinical conditions. Therefore, effective methods and new biomarkers will be required to diagnose prediabetes and diabetes. The results of IPA of up-regulated > 2-fold change of gene expression of miRNAs -29b-3p may be a very good therapeutic method for regulating the acute myocardial infarction, also regulates osteoblast differentiation via regulating IGF-1 [11,12]. Further, interaction network of miR-29b-3p is also generated using genes/molecules/pathways that have experimentally observed evidence of directly interacting with miR-29b-3p in people with T2DM **(Figure 9)**. There are eighteen (18) red up-regulated genes, four (4) green down-regulated and seven (7) no change genes (Figure 9). MicroR-548h-5p is a novel miRNA involved in protein synthesis, and various pathways [13], and mi-RNA-624-5p induces cell senescence in vitro, stop hepatoblastoma growth in vivo, and regulates the β-catenin 3´-untranslated region and it may be a very good candidate for miRNA replacement therapy for people with hepatoblastoma [14].

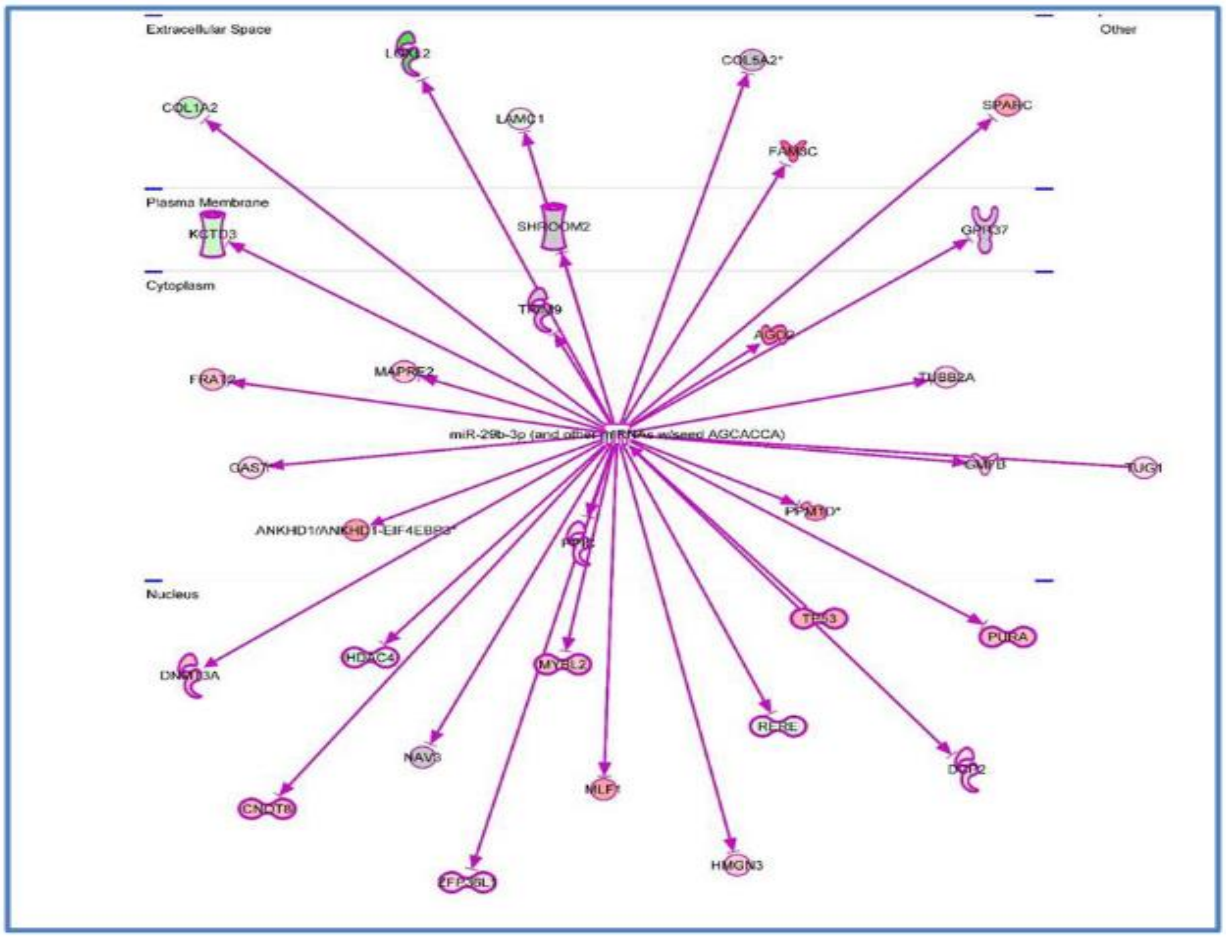


Figure 9: The interaction network of miR-29b-3p and other miRNAs w/seed AGGACCA.

MicroRNA-361-5p inhibits cancer progression by targeting signal transducer and activator of transcription 6 in non-small cell lung cancer and also inhibits cancer cell growth by regulating CXCR6 in hepatocellular carcinoma [15,16]. MicroRNA-130a-3p modulates activation and induces apoptosis of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis by directly acting on TGFBR1 and TGFBR2 via the TGF-β/SMAD signaling pathways [17]. It also regulates cell migration and invasion in gemcitabine resistant hepatoma cells as well as in nasopharyngeal carcinoma cells by inhibiting BACH2 expression [18,19]. MicroRNA-3912-3p is involved in the regulation of apoptosis, cell growth, cell cycle, inflammatory, lipid metabolism and oxidative stress response [20]. MicroRNA-1976 inhibits tumor and acts as a prognostic indicator in non-small cell lung cancer [21], and miRNA-1284 involves as a regulator to inhibit osteosarcoma cell proliferation and migration by targeting HMGB1 and also inhibits cell growth and induces apoptosis of lung cancer cells [22,23]. MicroRNA-3605-3p is differentially expressed genes in asthmatic bronchial epithelial cells [24].

Down-regulation of miRNA-324-3p induces promoter-mediated expression of ReIA gene and cleavage of caspase-3 on Ago2 [25]. MicroRNA-576-3p is a novel inhibitor of bladder cancer cell proliferation acting through targeting cyclin D1 [26]. MicroRNA-374c-5p inhibits the progression of breast cancer, modulate DEP containing 1 (DEPDC1) and

plays a pivotal role in cancers 6 [27]. MicroRNA-4326 is involved in chondrocyte apoptosis in osteoarthritis disease [28], and miRNA-5481 in dysregulation of key miRNAs in pancreatic cancer [29]. MicroRNA-4646-3p involves with epigenetic risk site of depression in adolescents [30], and miRNA-548ae-3p in several cancer diseases. MicroRNA-1247-3p modulates cartilage transcription through SOX9 factor [31]. MicroRNA-5695 regulates breast cancer and acts as its biomarker [32], and down-regulation of miRNBA-874-3p causes chemotherapeutic resistance in colorectal cancer via inactivation of Hippo signaling pathway [33].

All results of experimental design with respect to mRNA, miRNA, and paired mRNA-miRNA of IPA analyses data of gene expression profile of post-treatment has been described by Venn diagram, incorporating network images and canonical pathways as described in results. The network images indicate 9 mRNA, 10 miRNA and 29 overlap of paired mRNA-miRNA (29) as shown in **(Figure 10)** and Venn diagram of canonical pathways indicating 74 mRNAs, 23 miRNAs (23), and 174 paired mRNA-miRNA **(Figure 11).**

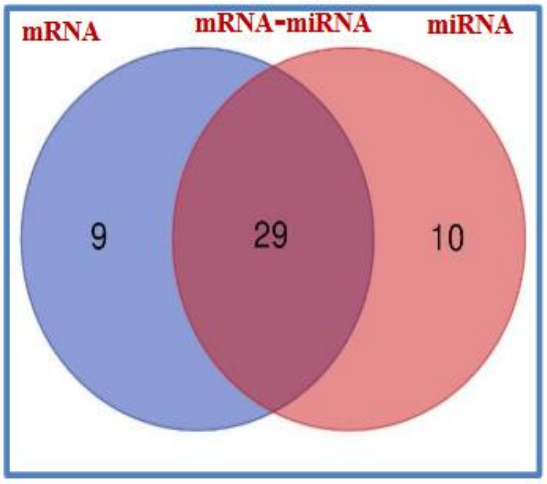


Figure 10: Venn Diagram-Network.

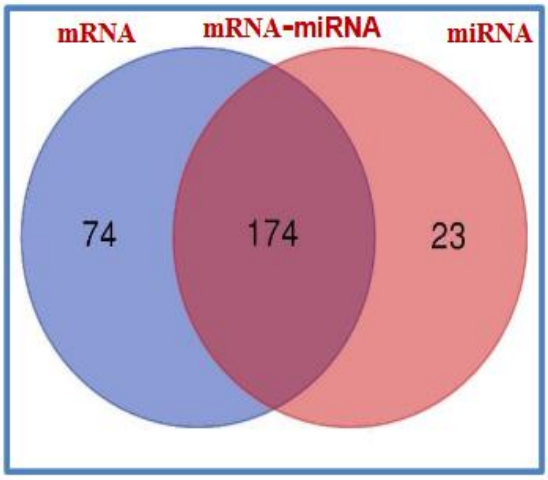


Figure 11: Venn Diagram Pathways.

As mentioned earlier, recent research focuses on the role of miRNAs in diabetes mellitus. There are different animal and tissue specific studies indicating the function of miRNAs as regulatory marker of glucose and lipid metabolism [34, 35]. The miRNA expression obtained from beta cells and tissues targeted by insulin altered in people with T2DM. This probably contributed to impaired function of these tissues under disease state [36]. MicroRNAs plays their role in beta cell differentiation, pancreatic beta cell development, glucose metabolism and insulin secretion by regulation of specific target genes like forkhead box protein A-2 (FoxA-2, also known as HNF-3 beta), myotrophin (Mtpn), pancreatic, duodenal homebox-1 (Pdx-1), inwardly rectifying potassium channel (Kir-6.2), and sulfonylurea receptor-1(Sur-1) [37].

The role of circulating miRNAs is gaining importance in disease mechanism as mentioned earlier due to their highly accessible, non-invasive marker with increased sensitivity and specificity [38]. Different tissue specific miRNAs found to be present in blood (miRNA 29a, miRNA 34a, miRNA 103, miRNA 107, miRNA 132, miRNA 144 and miRNA 375) [39]. They were detected in whole blood, serum/plasma. Here they showed greater stability against thawing and freezing cycles. Raised levels of miRNAs in circulation after tissue damage proved them possible markers of tissue injury [40,41]. The hypothesis that circulatory miRNAs might be acted as potential indicator of tissue level injury has been reported and has been demonstrated that after tissue injury, disease specific biomarkers adequately released into systemic circulation [41].

The characteristic expression profile of miRNAs in plasma of pre-diabetes or people with T2DM are reported recently, indicating lower plasma levels of miRNA-20b, miRNA-21, miRNA-24, miRNA-15a, miRNA-126, miRNA-191, miRNA-197, miRNA-223, miRNA-320, and miRNA-486 in prevalent diabetes [42]. The study has demonstrated that circulatory miRNAs as an initial predictor in T2DM. The results of another study showed miRNA-126 was significantly lower in individuals with diabetes compared to controls [43]. Moreover, the role of miRNA-126 in diabetic retinopathy established in mice with oxygen-induced retinopathy, its level significantly decreased due to retinal neovascularization by enhances the levels of VEGF, IGF-2 and HIF-1α through down-regulation of p38 and ERK signaling molecules [44].

Conclusions

The present study identifies the role of several miRNAs (miRNA-29b-3p, miR-624-5p, miR-361-5p, miR-130a-3p, miR-374-5p, miR-4326 [particularly for HbA1c], miR-1247-3p, miR-874-5p), which may predict and identify early onset of type 2 diabetes mellitus in humans. The overexpression of mRNA-AL1621513 indicates oxidative stress in people with T2DM, resulted in complications of diabetes, such as neuropathy, retinopathy, and stroke. These results look very good, but there are some limitations of using NGS analysis routinely now a days due to high cost of such analysis. It used to be $ 750 to $ 1000/RNA sample in the past, but now a days it cost $ 168 to 198/sample including data analysis. The cost of instrument varies from $ 800K to 1.5 million. Recently, GeoMx DSP ($ 100K) has been introduced to carry out limited number of genes 200 - 800, specific to a particular disease ($ 50/RNA sample plus data analysis). GeoMx DSP system can perform analysis up to 800 genes simultaneously, including miRNA, miRGE, IncRNA gene expression and fusion gene analysis. Recently RT-PCR technique is gaining importance in clinical laboratories to carry out samples of infected individuals. Therefore, estimation of various samples may be carried out by combination of GeoMax analysis plus RT-PCR assay, which may provide useful information to treat different diseases.

Acknowledgements:

*This article is a *Re-Print article of Journal of Diabetes and Clinical Studies, volume 5(1),* 2021, which has been ceased publication and has removed all their published articles from the Internet. **We thank Grace Pierce, Editorial Coordinator of *Journal of Clinical Surgery and Research* for creating the original title page of present article.** We also thank personals of Mayo Clinic, Rochester, MN., Mr. Chris P. Kolbert (Manger Research Operations), Mr. Robert A. Vierkant (Supervisor, section of Computational Genomics-Division of Biomedical Statistics and Informatic), Ms. Jane Kahl (Project Manager) for making arrangement of generating Venn diagrams and particularly, Ms. Mrunal K. Dehankar (Informatics Specialist) who has generated" Venn diagrams for the manuscript. We also thank "Mega Resveratrol" for supplying pure trans-resveratrol (99.9%, 60 Newton Road # 32, Danbury CT, USA. **Moreover, the input of my grandson (Mr. Ari A. Qureshi), patient, cooperation of Grace Pierce and Production Team of *Journal of Clinical Surgery and Research* are greatly appreciated.**

Funding:

The study was funded by Advanced Medical Research (AMR), Madison, Wisconsin, 53719, and in part by NIH funds 3452, 5RO1GM 102631, 3RO1GM 631S1, RO1 GM 50870 (N. Qureshi).

Conflicts of Interest:

All authors declare that they have no competing interests.

Author's Contributions:

AAQ, DAK, NQ conceived and was involved in planning the study. DAK and WM carried out human study and prepared total mRNAs, miRNAs for their sequencing. DPH has carried out RNA-sequence analyses, including data analyses. MKD has generated Venn diagrams. AAQ wrote the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials:

All data generated or analyzed during this study are included in this article.

Consent of Publication:

All contributing authors agree to the publication of this article.

Abbreviations:

T2DM = type 2 diabetes mellitus,

PBMC = peripheral blood mononuclear cells,

IRS-1 = insulin receptor substrate-1,

SOD-2 = superoxide dismutase-2,

GCKR = glucokinase regulators,

IGFBP-2 = insulin like factor binding protein-2,

IL-4 = interleukin-4,

IL-6 = interleukin-6,

iNOS = inducible nitric oxide,

IPA = Ingenuity Pathway Analysis.

References

1. Qureshi AA, Khan DA, Mahjabeen W, Silswal N, et al. (2021). Re-Print-A novel mixture of δ-tocotrienol, vitamin D_3, resveratrol (NS-3) significantly decreases diabetes biomarkers including inflammatory in people with type 2 diabetes. *Journal of Clinical Surgery and Research.* 5(2). [View Article]
2. Gardner DG, Shoback D (2011). Greenspan's basic & clinical endocrinology. 9th ed. New York: McGraw-Hill Medical pp. Chapter 17. [View Article]
3. American Diabetes association (ADA). Standards of medical care in diabetes-2011 *Diabetes care* 34(1): S11-S61. [View Article]
4. Esteller M (2011) Noncoding RNAs in human diseases. *Nature* 11: 861-874. [View Article]
5. Kevin C, Nikolaus R (2007). The evolution of gene regulation by transcription factors and microRNAs. *Nat Rev Genet* 8 (2): 93-103. [View Article]
6. Small EM, Olson EN (2001). Pervasive roles of microRNAs in cardiovascular biology. *Nature* 469: 336-342. [View Article]
7. Rong Y, Bao W, Shan Z, Liu J, et al. (2013). Increased microRNA-146a levels in plasma of patients with newly diagnosed type 2 diabetes mellitus. *PloS One* 8(9): e73272. [View Article]
8. Balasubramanyam M, Aravind S, Gokulakrishnan K, Pramasivam P, et al. (2011). Impaired miR-146a expression links subclinical inflammation and insulin resistance in type 2 diabetes. *Mol Cell Biochem* 351(1-2): 197-205. [View Article]
9. Duan X, Zhan Q, Song B, Zeng S, et al. (2014). Detection of platelet microRNA expression in patients with diabetes mellitus with or without ischemic stroke. *J Diabetes Complications* 28(5): 705-710. [View Article]
10. Xu J, Wu W, Zhang L, Dorset-Martin W, et al. (2012). The Role of microrna-146a in the pathogenesis of the diabetic wound-healing impairment correction with mesenchymal stem cell treatment. *Diabetes* 61: 2906-2912. [View Article]
11. Cai Y, Li Y (2019). Upregulation of miR-29b-3 protects cardiomyocytes from hypoxia-induced apoptosis by targeting TRAF5. *Cellular and Molecular Biology Letters* 24:27. [View Article]
12. Zeng Q, Wang Y, Gao J, Yan Z, et al. (2019). miR-29b-3p regulated osteoblast differentiation via regulating IGF-1 secretion of mechanically stimulated osteocytes. *Cellular and Molecular Biology Letters* 24:11. [View Article]
13. Liang T, Guo L, Liu C. (2012) Genome-wide analysis of mir-548 gene family reveals evolutionary and functional implication. *Journal of Biomedicine and Biotechnology*. Article ID 679563, 8 pages. [View Article]
14. Indersie, E, Lesjean S, Hooks KB, Sagliocco F, et al. (2017). Micro RNA therapy inhibits hepotoblastoma growth in vivo by targeting β-catenin and Wnt signaling. *Hepatology Communications* 1(2):168-183. [View Article]
15. Ma Y, Bao C, Kong R, Xing X., et al. (2015). MicroRNA-361-5p suppress cancer progression by targeting signal transducer and activator of transcription 6 in non-small cell lung cancer. *Molecular Medicine Reports* 12:7367-7373. [View Article]
16. Sun JJ, Chen GY, Xie ZT. (2016). Micro-RNA-361-5p inhibits cancer cell growth by targeting CXCR6 in hepatocellular carcinoma. *Cellular Physiology and Biochemistry* 38:777-785. [View Article]
17. Wang Y, Du J, Niu X, Fu N, et al. (2017). MiR-130a-3P attenuates activation and induces apoptosis of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis by directing targeting TGFBR1 and TGFBR2. *Cell Death and Disease* 8: e2792. [View Article]
18. Liu Y, Li Y, Wang R, Qin S, et al. (2016). MiR-130a-3p regulates cell migration and invasion via inhibition of Smad4 in gemcitabine resistant hepatoma cells. *Journal of Experimental & Clinical Cancer Research* 35:19. [View Article]
19. Chen X, Yue B, Zhang C, Qi M, et al. (2017). MiR-130a-3p inhibits the viability, proliferation, invasion, and cell cycle, and promote apoptosis of nasopharyngeal carcinoma cells by

suppressing BACH2 expression. *Bioscience Reports* 37: BSR20160576. [View Article]
20. Shen Y, Liu M, Chen C, Lai X, et al. (2017). MicroRNA profile changes in liraglutide treated steatotic HepG2 cells. *Int.J. Exp Med* 10(3): 4856-4864. [View Article]
21. Chen G, Hu J, Huang Z, Yang L, et al. (2016). MicroRNA-1976 functions as a tumor suppressor and serves as a prognostic indicator in non-small cell lung cancer by directly targeting PLCE1. *Biochem Biophysic Res Commun* 473 (4): 1144-1151. [View Article]
22. Lv S, Guan M (2018). miRNA-1284, a regulator of HMGB1, inhibits cell proliferation and migration in osteosarcoma. *Bioscience Reports* 38: BSR20171675. [View Article]
23. Li J, Jin H, Yu H, Wang B, et al. (2017). miRNA-1284 inhibits cell growth and induces apoptosisof lung cancer cells. *Molecular Medicine Reports* 16: 3049-3054. [View Article]
24. Alexandrova E, Miglino N, Hashim A, Nassa G, et al. (2016). Tamm M. Small RNA profiling reveals deregulated phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K)/Akt pathway in bronchial smooth muscle cells from asthmatic patients. *Journal of Allergy and Clinical Immunology* 137(1): 58-67. [View Article]
25. Dharap A, Pokrzwa C, Murali S, Pandi G, et al. (2013). MicroRNA miR-324-3p induces promoter-mediated expression of ReIA gene. *PloS One* 811: e79467. [View Article]
26. Liang Z, Li S, Zu X, Xu X, et al. (2015). MicroRNA-576-3p inhibits proliferation in bladder cancer cells by targeting cyclin D1. *Molecules and Cells* 38(2): 130-137. [View Article]
27. Unterbruner K, Matthes F, Schilling J, Nalavade R, et al. (2018). MicroRNAs miR-19, miR-340, miR-374 and miR-542 regulate MID1 protein expression. *PloS One* 13(1): e0190437. [View Article]
28. Lee EC, Valencia T, Allerson C, Schairer A, et al. (2019). Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease. *Nature Communications* 10: 4148. [View Article]
29. Baradaran B, Shahbazi R, Khardadmehr M (2019). Dysregulation of key microRNAs in pancreatic cancer development. *Biomedicine & Pharmacotherapy* 109: 1008-1015. [View Article]
30. Bostrom AE, Ciuculete D-M, Attwood M, Krattinger R, et al. (2017). A miR-4646 associated methylation locus is hypomethylated in adolescent depression. *Journal of Affective Disorder* 220: 117-128. [View Article]
31. Martinez-Sanchez A, Murphy C.L (2013). miR-1247 functions by targeting cartilage transcription factor SOX9. The *Journal of Biological Chemistry* 288(43): 3082-30814. [View Article]
32. Pallares-Albanell J, Zomeno-Abellan MT, Escaramis G, Pantano L, et al. (2019). A high-throughput screening identifies microRNA inhibitors that influence neuronal maintenance and/or response to oxidative stress. *Molecular Therapy Nucleic acids* 17:374-387. [View Article]
33. Que K, Tong Y, Que G, LI L, et al. (2017). Down regulation of miR-874-3p promotes chemotherapeutic resistance of the Hippo signaling pathways. *Oncology Reports.* 38: 3376-3386. [View Article]
34. Herrera BM, Lock-stone HE, Taylor JM, Ria M, et al. (2010). Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes. *Diabetologia* 53: 1099-1109. [View Article]
35. Zhao E, Keller MP, Rabaglia ME, Oler AT, et al. (2009). Obesity and genetics regulate microRNAs in islets, liver, and adipose of diabetic mice. *Mamm Genome* 20: 476-485. [View Article]
36. Shantikumar S, Caporali A, Emanuell C (2019). Role of miRNAs in diabetes and its cardiovascular complications. *Cardiovasc Res* 93: 583-593. [View Article] [View Article]
37. Tang X, Tang G, Özcan S. (2008) Role of microRNAs in diabetes. *Biochim Biophys Acta* 11779 (11): 697-701.46. [View Article]
38. Creemers EE, Tijsen AJ, Pinto Y.M (2012). Circulatory microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Cir Res* 110: 483-495. [View Article]
39. Gilad S, Meiri E, Yogev Y, Benjamin S, et al. (2008). Serum microRNAs are promising novel biomarkers. *PloS One* 3: e3148. [View Article]
40. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, et al. (2011). MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 13(4): 423-433. [View Article]
41. Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, et al. (2009). Plasma micro RNAs as sensitive and specific biomarkers of tissue injury. *Clin Chem* 55: 1977-1983. [View Article]
42. Zampetaki A (2010). Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ. Res* 107: 810-817. [View Article]
43. Liu y, Gao G, Yang C, Zhou K, et al. (2014). The role of circulating microRNA-126 (miR-126): a novel biomarker for screening prediabetes and newly diagnosed type 2 diabetes mellitus. *Int J Mol Sci* 15: 10567-10577. [View Article]
44. Bai Y, Bai X, Wang Z, Zhang X, et al. (2011). MicroRNA-126 inhibits ischemia-induced retinal neovascularization via regulating angiogenic growth factors. *Exp Mol Pathol* 91: 471-477. [View Article]

DOI: 10.31579/2768-2757/118

Supplement File

Table 9A: IPA analysis of mRNAs associated with "up-stream" gene exression (up-regulated [42]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Up-stream Regulators	Expression Log Ratio	Molecule Type	Predicted Activation State	Activation z-score	Flags	*P*-value of overlap	Target molecules	Mechanistic Network
1	TNF		cytokine	Activated	7.5	bias	1.56E-11	ACADVL,ACSL1,ADAM8,A	457 (14)
2	IL-5		cytokine	Activated	7.2	bias	4.25E-10	ACAA2,ALDOA,ANXA2,AN	658 (19)
3	IL-1B		cytokine	Activated	6.7	bias	0.000000154	ADAM8,AIF1,AKR1B1,ALP	579 (17)
4	STAT1		transcription regulator	Activated	5.9	bias	0.0000124	APOBEC3G,B2M,BAX,BST2	615 (17)
5	Interferon alpha		group	Activated	5.9	bias	5.58E-14	ANXA5,APOBEC3G,B2M,B(	477 (15)
6	IL-6		cytokine	Activated	5.8	bias	2.83E-08	ADGRE5,AHNAK,ANPEP,A	647 (16)
7	STAT3		transcription regulator	Activated	5.8	bias	4.28E-13	AHSP,APEX1,BATF,BCL3,B	604 (20)
8	IL-4		cytokine	Activated	5.3	bias	3.49E-18	ABCA2,ACSL1,ACTG1,ADA	446 (17)
9	IL-2		cytokine	Activated	4.8	bias	3.47E-19	ADAM8,ANXA1,APOBEC3(	618 (18)
10	ATF4	2.093	transcription regulator	Activated	4.6	bias	0.00348	APEX1,ATF4,CALR,CANX,(	621 (19)
11	IL-1A		cytokine	Activated	4.3	bias	0.0226	BAX,BCL3,CCL4,CCL5,CD4	
12	CCL5	2.342	cytokine	Activated	4.1	bias	9.73E-08	ADGRE5,AIF1,ATF4,C5AR1	385 (15)
13	IL-15		cytokine	Activated	4.1	bias	1.53E-32	ACTN4,ALDOA,ANXA1,AR	700 (20)
14	IL-27		cytokine	Activated	4.0	bias	0.000195	B2M,BST2,CCL4,CCL5,CCN	664 (19)
15	CXCL12		cytokine	Activated	4.0	bias	3.17E-10	AIF1,BAX,BCL3,CANX,CCL	558 (16)
16	IL-12 (complex)		complex	Activated	3.9	bias	0.00000121	BCL3,CASP1,CCL4,CCL5,CC	420 (16)
17	EIF4E		translation regulator	Activated	3.7	bias	0.000696	CAPZB,CEBPB,DEDD2,EIF4	
18	MAPK14		kinase	Activated	3.5	bias	0.0357	ALOX5AP,BAX,CALR,CAT,	
19	AKT1		kinase	Activated	3.4	bias	0.00461	AIP,ARL6IP1,ATP5MC1,AT	666 (20)
20	PTGS2		enzyme	Activated	3.2	bias	0.0102	ANXA1,ANXA2,BAX,CCL4,	
21	IL-7		cytokine	Activated	3.2	bias	0.000446	BAX,CCL4,CCND3,CD2,CD2	465 (17)
22	PIK3R1		kinase	Activated	2.9	bias	0.00169	ATF4,CCL4,CDKN1B,FOS,H	558 (18)
23	IL-13		cytokine	Activated	2.9	bias	0.0000082	ACADVL,ACSL1,ADAM8,A	583 (16)
24	CD2	2.831	transmembrane receptor	Activated	2.8	bias	0.000164	CCR7,CD4,CD44,CD48,CD8A	337 (17)
25	ATF6		transcription regulator	Activated	2.7	bias	0.00162	CALR,CEBPB,FOS,HSP90B1	570 (20)
26	PIK3CA		kinase	Activated	2.6		0.0215	CD14,CD44,CDKN1B,CEBPI	
27	IL-10		cytokine	Activated	2.5		0.000000297	ALOX5AP,BAX,BCL3,CAM	627 (19)
28	AKT3		kinase	Activated	2.4		0.00215	CDKN1B,COX4I1,ETS1,MT-	
29	CAPN3		peptidase	Activated	2.4	bias	0.000434	CCL5,DUSP1,FTH1,HMOX1	242 (7)
30	CCL3		cytokine	Activated	2.4	bias	0.00815	APOBEC3G,CCL4,FOS,ITGA	440 (15)
31	STAT2		transcription regulator	Activated	2.2	bias	0.00389	CCL5,IFI27,IFI35,IFITM1,IFI	405 (11)
32	IL-18		cytokine	Activated	2.2	bias	0.000756	CAMP,CCL4,CCL5,CCR7,CD	608 (19)
33	IL-12B		cytokine	Activated	2.2	bias	0.0101	CCL5,CDKN2D,CEBPD,GZM	
34	MAPK8		kinase	Activated	2.2	bias	0.000312	ATG7,CAT,CCL5,CDKN1B,(	611 (18)
35	CCL-34		chemical reagent	Activated	2.0	bias	0.00159	CD4,CD8A,ITGAM,ITGAX	
36	CXCL3		cytokine		2.0	bias	0.0386	CCL4,CCL5,GRK2,ITGB1	
37	IL-24		cytokine		1.5	bias	0.00854	BAX,CASP8,EIF2A,HSP90B1	566 (18)
38	ATP		chemical - endogenous m		1.4	bias	0.00644	BAX,CAPN1,CAPN2,CASP1	395 (17)
39	ATF1		transcription regulator		1.3	bias	0.0103	CEBPB,FOS,FTH1,HLA-B,H	
40	CXCL8		cytokine		1.2	bias	0.00587	ANXA1,BAX,CD44,CD74,CX	612 (18)
41	Cdc42		enzyme		1.0	bias	0.000486	BID,CAPZA1,CASP1,CTSH,(	200 (7)
42	MAPT		other		0.8		1.1E-18	ACTG1,ADD1,AIF1,ALDOA	534 (9)

Table 9B: IPA analysis of mRNAs associated with "up-stream" gene exression (down-regulated [26]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Upstream Regulators	Expression Log Ratio	Molecule Type	Predicted Activation State	Activation z-score	Flags	*P*-value of overlap	Target molecules in dataset	Mechanistic Network
43	miR-124-3p (and other		mature microRNA	Inhibited	-5.8	bias	0.0000662	ACAA2,ARHGEF1,ARPC1B,	
44	mir-21		microRNA	Inhibited	-5.8	bias	0.00000035	AIF1,ARF4,ARF6,ARL6IP1,E	418 (14)
45	miR-16-5p (and other m		mature microRNA	Inhibited	-4.9	bias	0.00854	ANAPC16,CAPRIN1,CCND3	
46	miR-1-3p (and other mi		mature microRNA	Inhibited	-4.6	bias	0.000163	ANP32B,ANPEP,ANXA2,AF	
47	miR-155-5p (miRNAs v		mature microRNA	Inhibited	-4.2	bias	0.000387	ATG3,CASP1,CCL4,CD47,CE	420 (10)
48	IL-1RN		cytokine	Inhibited	-4.1	bias	0.0133	CD44,CTSS,GSDMD,IFI27,IL	
49	mir-122		microRNA	Inhibited	-3.9	bias	0.0000235	ALDOA,CCNG1,CDA,CUX1	
50	mir-10		microRNA	Inhibited	-3.7	bias	0.00522	ALOX5,BAX,CD44,DGAT1,	444 (12)
51	mir-155		microRNA	Inhibited	-3.6	bias	0.000357	ANXA2,CCL5,CD47,CD68,C	555 (17)
52	mir-210		microRNA	Inhibited	-3.4	bias	0.042	CHD9,DDOST,FCER2,IL16,K	
53	mir-1		microRNA	Inhibited	-3.3	bias	0.0156	ADD1,ANP32B,ANXA2,CAI	
54	miR-30c-5p (and other r		mature microRNA	Inhibited	-3.1	bias	0.0156	ANPEP,AP2A1,CHD1,FRG1,	
55	miR-200b-3p (and other		mature microRNA	Inhibited	-2.6	bias	0.0356	BST1,CDKN1B,HDAC3,MA1	
56	mir-25		microRNA	Inhibited	-2.4	bias	0.0309	BAX,ITGA5,MCL1,MDM2,E	
57	mir-193		microRNA	Inhibited	-2.4	bias	0.0269	ACTN4,CD151,MCL1,PTEN,	
58	MAPK1		kinase	Inhibited	-2.3		0.00000281	ANPEP,BST2,CAPN2,CCL4,(	490 (17)
59	ABCC8		transporter	Inhibited	-2.2	bias	0.0062	ACADVL,CAT,MT-CO1,MT	
60	miR-124		group	Inhibited	-2.1	bias	0.00268	IQGAP1,PTPRC,RELA,RHO(	
61	mir-154		microRNA		-2.0	bias	0.0205	BAX,CDKN1B,HMOX1,PTE	
62	mir-193a-3p (and other		mature microRNA		-2.0	bias	0.00627	ETS1,MCL1,RPS6KB2,SRSF2	214 (7)
63	mir-8		microRNA		-1.9	bias	0.00675	BAX,BST1,CD47,CDC25B,C	552 (15)
64	mir-124		microRNA		-1.5	bias	0.0327	BAX,CAMTA1,IQGAP1,PTE	
65	mir-148		microRNA		-1.3	bias	0.000722	DNMT1,IKZF1,PKM,PTEN,	
66	Akt	as are	group		-0.7	bias	0.00371	ATF4,ATG7,ATP5F1A,BAX	513 (15)
67	PSMD10		transcription regulator		-0.2	bias	0.0278	BAX,CDKN1B,EIF4EBP1,GN	
68	IL-2RG	2.329	transmembrane receptor		0.0		0.0000309	ARHGAP45,CCND3,COL6A1	382 (17)

The "up-stream regulators" indicates 42 up-regulated and 28 down-regulated (Tables 9A & 9B) out of total 1300. Gene expression of all types of cytokines are up-regulated, except IL-1RN and IL-2RG. Whereas, various type of miRNAs are down-regulated which plays important roles modulating in T2DM as described in results. The important up-stream regulators are TNF-α, IL-5, IL-1B, STAT1, IL-2, IL-4, IL-6, ARF4, EIF4B, MAPK14, CD2 and interferon-a. Whereas, various types of miRNAs and mir- are down-stream regulators, which plays important roles modulating in T2DM are described in the results. Most of the down-stream regulators belong to mir- group (mir-21, mir-122, mir-10, mir-210 etc, rather than miR-16-5p and miR-124-3p).

Table 10A: IPA analysis (mRNAs) of gene expression of "diseases functions" (up-regulation [31]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Categories	Diseases or Function Annotation	*P*-value	Predicted Activation State	Activation z-score	Molecules	Number of Molecules
1	Inflammatory Response	Degranulation	4.67E-82	Increased	3.0	ACAA1,ACTN4,ACTR10,ACTR	237
2	Cellular Compromise,Inflammatory Response	Degranulation of neutrophils	4.07E-76	Increased	2.1	ACAA1,ACTR10,ACTR2,ADAM	176
3	Cellular Compromise,Inflammatory Response	Degranulation of granulocytes	7.78E-76	Increased	2.9	ACAA1,ACTR10,ACTR2,ADAM	178
4	Infectious Diseases	Viral Infection	3.71E-66	Increased	8.6	ACSL1,ACTR2,ACTR3,ADGRE5	406
5	Immunological Disease	Systemic autoimmune syndrome	3.47E-42	Increased	2.0	ACSL1,ADAM8,ADGRE5,AES,A	311
6	Cellular Movement	Cell movement of blood cells	8.96E-38	Increased	7.2	ACTN4,ADAM8,ADGRE5,AIF1	249
7	Cellular Movement,Immune Cell Trafficking	Leukocyte migration	9.64E-37	Increased	7.1	ACTN4,ADAM8,ADGRE5,AIF1	245
8	Cellular Development,Cellular Growth and Prol	Proliferation of mononuclear leukocytes	1.12E-36	Increased	3.5	AHNAK,AIF1,AKIRIN2,ANPEP	203
9	Cellular Function and Maintenance	Engulfment of cells	6.19E-34	Increased	6.5	ACTN4,ADD1,ANPEP,ANXA1,	141
10	Inflammatory Response	Immune response of cells	8.99E-33	Increased	4.9	ANPEP,ANXA1,ANXA3,ANXA	163

11	Infectious Diseases	Infection by RNA virus	1.27E-29	Increased	9.8	ACTR2,ACTR3,ADGRE5,AES,A	211
12	Cellular Function and Maintenance	Endocytosis	1.75E-27	Increased	6.3	ACTG1,ACTN4,ADD1,ANK1,A	147
13	Cell Death and Survival	Cell survival	3.28E-26	Increased	10.5	ACTN4,AGTRAP,AKAP8L,ANX	300
14	Free Radical Scavenging	Synthesis of reactive oxygen species	4.97E-26	Increased	3.9	AHSP,AKR1B1,ALOX5,ANXA1	133
15	Cellular Movement,Immune Cell Trafficking	Cell movement of lymphatic system cells	9.78E-25	Increased	5.2	ACTN4,ADAM8,ALOX5,ANXA	121
16	Cell-To-Cell Signaling and Interaction,Hematolo	Binding of leukocytes	1.2E-23	Increased	7.1	ADGRE5,ANXA1,ANXA5,APBI	108
17	Cell-To-Cell Signaling and Interaction,Hematolo	Adhesion of immune cells	2.31E-23	Increased	7.0	ADGRE5,ANXA1,ANXA5,APBI	102
18	Gene Expression	Expression of RNA	5.47E-23	Increased	3.8	ABCA2,ABHD14B,ACTN4,ACT	397
19	Free Radical Scavenging	Production of reactive oxygen species	2.58E-20	Increased	3.2	AHSP,AKR1B1,ALOX5,ANXA1	100
20	Cell Death and Survival	Apoptosis of leukocytes	5.21E-18	Increased	0.6	ADAM8,AKIRIN2,ANPEP,ANX	110
21	Cell-To-Cell Signaling and Interaction,Hematolo	Adhesion of mononuclear leukocytes	1.88E-16	Increased	5.5	ANXA1,APBB1IP,ARHGEF1,CC	49
22	Cellular Assembly and Organization	Development of cytoplasm	2.06E-16	Increased	5.0	ACTR2,ACTR3,ADD1,AIF1,AK	116
23	Cell-To-Cell Signaling and Interaction	Adhesion of lymphatic system cells	2.23E-16	Increased	5.3	ANXA1,ANXA2,ANXA2R,APB	45
24	Cell-mediated Immune Response,Cellular Funct	T cell homeostasis	4.78E-16	Increased	5.7	ANXA1,B2M,BATF,BAX,BCL3	122
25	Cellular Function and Maintenance	Homeostasis of blood cells	8.26E-16	Increased	6.1	ANXA1,B2M,BATF,BAX,BCL3	129
26	Cell-mediated Immune Response,Cell-To-Cell S	Adhesion of T lymphocytes	6.83E-15	Increased	4.5	ANXA1,APBB1IP,ARHGEF1,CC	37
27	Cell Death and Survival,Cellular Compromise	Cytotoxicity of lymphocytes	8.78E-15	Increased	4.5	B2M,CCL5,CD2,CD247,CD300A	47
28	Antimicrobial Response,Inflammatory Respons	Antimicrobial response	9.04E-13	Increased	2.1	ANXA3,APOBEC3G,ATG7,BAN	76
29	Cell Cycle,Gene Expression	Binding of DNA	1.51E-12	Increased	1.8	ALOX5,ANXA7,APEX1,BAZ2A	106
30	Cell-To-Cell Signaling and Interaction	Aggregation of cells	1.19E-11	Increased	5.6	AKR1B1,ANXA7,ATG7,ATP2A	73
31	Cellular Movement,Hematological System Deve	Chemotaxis of mononuclear leukocytes	5.01E-10	Increased	3.6	ADAM8,AIF1,ANXA1,CAMP,C	45

Table 10B: IPA analysis (mRNAs) of gene expression of "diseases functions" (down-regulation [14]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Categories	Diseases or Functions Annotation	*p*-value	Predicted Activation State	Activation z-score	Molecules	Number of Molecules
32	Infectious Diseases	Parasitic Infection	1.91E-09	Decreased	-3.4	AHNAK,ANXA1,B2M,BCL3,C5	43
33	Cell Death and Survival	Necrosis	3.69E-43	Decreased	-2.9	ACSL1,ADAM8,ADRM1,AGPA	523
34	Cancer,Hematological Disease,Immunological D	T-cell non-Hodgkin lymphoma	3.82E-09	Decreased	-2.2	ANXA1,BAX,BTG1,CAMLG,CC	88
35	Cell Death and Survival	Cell death of tumor cell lines	8.65E-30	Decreased	-2.1	ADRM1,AGPAT2,AHSA1,AKR	328
36	Cancer,Organismal Injury and Abnormalities	Solid tumor	9.37E-29	Decreased	-2.1	AAMP,ABCA2,ABHD14B,ABH	1471
37	Cancer,Organismal Injury and Abnormalities	Non-hematological solid tumor	5.64E-28	Decreased	-2.8	AAMP,ABCA2,ABHD14B,ABH	1443
38	RNA Post-Transcriptional Modification	Splicing of RNA	1.14E-22	Decreased	-2.1	AHNAK,C1QBP,CASC3,CCAR2	79
39	Cancer,Cell Death and Survival,Organismal Inju	Cell death of osteosarcoma cells	1.12E-21	Decreased	-6.9	COPG1,EEF1A1,EIF3F,KPNB1,N	48
40	RNA Post-Transcriptional Modification	Splicing of mRNA	1.38E-21	Decreased	-3.1	C1QBP,CASC3,CPSF1,CTNNBL	71
41	Cancer,Cell Death and Survival,Organismal Inju	Necrosis of tumor	1.55E-20	Decreased	-3.3	ANPEP,ANXA1,ANXA2,ATG7,	116
42	Cancer,Cell Death and Survival,Organismal Inju	Necrosis of malignant tumor	2.91E-20	Decreased	-3.9	ANPEP,ANXA1,ATG7,B2M,BA	101
43	Gene Expression,Protein Synthesis	Translation of mRNA	4.46E-17	Decreased	-2.9	BANK1,BTG2,CALR,CAPRIN1,	55
44	Organismal Survival	Morbidity or mortality	1.52E-14	Decreased	-15.0	ABCA2,ACADVL,ACRBP,ACTC	393
45	Hematological Disease,Immunological Disease	Leukopenia	2.05E-14	Decreased	-1.0	B2M,BATF,C5AR1,CAMLG,CD	51

The "diseases function" (out of 500) shows 31 up-regulated are involved in inflammatory response, infectious diseases, immunological diseases, cellular functions, its maintenance, cellular death and survival. Whereas, 14 down-regulated gene expression are mainly associated with cancer, organismal injury, abnormalities, RNA post-transcriptional modification and protein synthesis.

Table 11: Summary of IPA analysis of mRNA for "Networks" and "top diseases and functions" (25 each out of 500 genes) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	mRNA - Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
1	ACADVL,ATP5MD,ATP5PD,BCKDHA,C1QBP,CH	42	34	[Developmental Disorder, Hereditary Disorder, Metabolic Disease]
2	ARF1,CHTOP,COP I,COPE,COPG1,COPZ1,CRIP2,D	40	33	[Cellular Assembly and Organization, Infectious Diseases, Small Molecule Biochemistry]
3	ABI3,ANXA2R,CAPZA1,CAPZB,CNOT1,CNOT2,CI	40	33	[Cell Signaling, Post-Translational Modification, RNA Post-Transcriptional Modification]
4	60S ribosomal subunit,BANF1,BCL7C,BRD9,CNBP,Ei	40	33	[Cancer, Protein Synthesis, RNA Damage and Repair]
5	ADCY,ATIC,B3GAT3,DAZAP1,GAA,GSPT1,hexokir	40	33	[Hereditary Disorder, Protein Synthesis, RNA Post-Transcriptional Modification]
6	ANKRD13D,ARL6IP1,BTF3,CAMTA2,COX5A,CRT	37	32	[DNA Replication, Recombination, and Repair, Gene Expression, RNA Post-Transcriptional Modification]
7	adenosine-tetraphosphatase,AHSP,ARL4C,ATP5F1A,A	37	32	[Cellular Compromise, Energy Production, Inflammatory Response]
8	AGTRAP,CAMLG,CARS2,CK1ε/CK1δ,CMTM5,CO	35	31	[Cardiovascular Disease, Cell-To-Cell Signaling and Interaction, Cellular Movement]
9	ACAA1,ACAA2,acetyl-CoA C-acyltransferase,APOBR	35	31	[Cell Signaling, Post-Translational Modification, Protein Synthesis]
10	ANAPC15,ANAPC16,ANKRD44,B4GALT7,BUB3,C	35	31	[Molecular Transport, RNA Post-Transcriptional Modification, RNA Trafficking]
11	14-3-3(β,γ,θ,η,ζ),amylase,ARL6IP5,CAPRIN1,CAST,C	35	31	[Infectious Diseases, Organismal Injury and Abnormalities, RNA Post-Transcriptional Modification]
12	AKR1A1,Alpha 1 antitrypsin,ANXA7,CMTM3,COPS	35	31	[Dermatological Diseases and Conditions, Developmental Disorder, DNA Replication, Recombination, and Repair]
13	ABHD17A,AKR1B1,ARF6,Beta Arrestin,C4orf3,CALI	35	31	[Cell-mediated Immune Response, Cellular Movement, Hematological System Development and Function]
14	Aconitase,ANK1,ASNA1,AURK,BANK1,BLOC1S1,C	33	30	[Cell Cycle, Cell Death and Survival, Post-Translational Modification]
15	ACTR2,ACTR3,AES,ALDOA,C20orf27,CARHSP1,CC	33	30	[Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Reproductive System Development and Function]
16	ANP32B,BBIP1,BRD4,BTN3A2,CALR,caspase,CCAF	33	30	[Cancer, Cell Cycle, Embryonic Development]
17	Anti-inflammatory Cytokine,ATG,ATG16L2,ATG3,C1	31	29	[Dermatological Diseases and Conditions, Inflammatory Disease, Protein Synthesis]
18	ANP32E,ARL6IP4,CCNG1,CD164,CLASRP,EMC6,en	31	29	[Cellular Development, Cellular Growth and Proliferation, Lipid Metabolism]
19	AHSA1,CHD3,Ciap,IKK (complex),LYAR,NIK,NOP5	31	29	[Cancer, Protein Synthesis, RNA Damage and Repair]
20	Ap1,APEX1,ARGLU1,ATXN2L,CHD4,DDX17,ERC	31	29	[Cell Cycle, Cellular Development, DNA Replication, Recombination, and Repair]
21	AAMP,alcohol group acceptor phosphotransferase,Casp	27	27	[Developmental Disorder, Embryonic Development, Post-Translational Modification]
22	ANXA3,ARAP1,ARHGAP45,BCR (complex),C12orf7	27	27	[Cell Death and Survival, Cellular Development, Cellular Growth and Proliferation]
23	ABHD14B,ADGRG1,APRT,ASAH1,ATP2A3,CCL5,C	27	27	[Cellular Function and Maintenance, Cellular Growth and Proliferation, Hematological System Development and Function]
24	Adaptor protein,Adaptor protein 1,AP-3,Ap1 gamma,A	26	26	[Amino Acid Metabolism, Developmental Disorder, Post-Translational Modification]
25	APC/APC2,Casein,CHCHD10,CIAO2B,CK1,CMC1,C	26	26	[Developmental Disorder, Hereditary Disorder, Metabolic Disease]

The top diseases and functions indicate 25 out of 500 genes associated with T2DM. The important one are developmental disorder, hereditary disorder and metabolic diseases.

Table 12: IPA analysis of mRNA for "Ingenuity Canonical signaling Pathways" (51) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Ingenuity Canonical Pathways	Exp-log (*P*-values)	Ratio	z-score	Molecules
1	EIF2 Signaling	35.2	0.4	5.1	ATF4,EIF1,EIF2A,EIF2AK4,EIF2S3,EIF3D,EIF
2	Mitochondrial Dysfunction	26.3	0.4	#NUM!	ATP5F1A,ATP5F1B,ATP5F1E,ATP5MC1,ATP
3	Oxidative Phosphorylation	25.6	0.4	6.9	ATP5F1A,ATP5F1B,ATP5F1E,ATP5MC1,ATP
4	Sirtuin Signaling Pathway	19.5	0.2	-1.7	AGTRAP,APEX1,ATG16L2,ATG3,ATG7,ATP
5	mTOR Signaling	19.4	0.3	3.5	CDC42,DGKZ,EIF3D,EIF3F,EIF3H,EIF3I,EIF3M
6	IL-8 Signaling	11.3	0.2	5.5	BAX,CCND3,CDC42,CXCR1,DEFA1 (includes
7	Protein Ubiquitination Pathway	11.1	0.2	#NUM!	B2M,DNAJA1,DNAJB1,DNAJB6,DNAJC1,DN
8	Cdc42 Signaling	11.0	0.2	4.2	ACTR2,ACTR3,ARPC1A,ARPC1B,ARPC3,ARI
9	T Cell Receptor Signaling	7.6	0.2	#NUM!	CALM1 (includes others),CD247,CD3D,CD3E,C
10	Cytotoxic T Lymphocyte-mediated Apoptosis of T	7.5	0.4	3.0	B2M,BID,CASP8,CD247,CD3D,CD3E,CD3G,F
11	Interferon Signaling	7.1	0.4	3.1	BAX,IFI35,IFITM1,IFITM2,IFITM3,IFNGR1,I
12	Natural Killer Cell Signaling	7.1	0.2	#NUM!	CD247,CD300A,FCER1G,FCGR2A,FCGR3A/F
13	Th1 and Th2 Activation Pathway	6.6	0.2	#NUM!	CD247,CD3D,CD3E,CD3G,CD4,CD8A,CHD4,C
14	Type I Diabetes Mellitus Signaling	6.6	0.2	3.6	BID,CASP8,CD247,CD3D,CD3E,CD3G,FCER1

Table 13A: IPA analysis of paired (miRNA - mRNA) gene expression of "upstream regulators" (up-regulated [32]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

	Genes	Expression	Molecule Type	Predicted	Activation	Flags	*P*-value	Target Molecules in data set	Mechanistic
#		Log Ratio		Activation State	z-zone		overlap		Network
1	TNF-α		cytokine	Activated	6.1	bias	2.28E-10	ACADVL,ACSL1,ADAM8,AKR1B1,A	232 (16)
2	IL-1B		cytokine	Activated	5.8	bias	0.00000013	ADAM8,AKR1B1,ALPL,ARL6IP5,AT	275 (19)
3	NF-κB (complex)		complex	Activated	4.2	bias	0.00000237	ATP6AP2,BAX,BID,CASP8,CCL4,CCI	261 (16)
4	I-L4		cytokine	Activated	4.0	bias	6.64E-14	ACSL1,ACTG1,ADAP1,AHNAK,ALO	221 (17)
5	IL-6		cytokine	Activated	3.8	bias	1.7E-09	AHNAK,ANPEP,ARL4C,BAX,BTG2,E	215 (18)
6	IL-2		cytokine	Activated	3.7	bias	3.28E-11	ADAM8,ARHGEF2,BAX,CCL4,CCL5,	248 (18)
7	CCL5	2.342	cytokine	Activated	3.7	bias	0.000000165	ATF4,C5AR1,CCL4,CCL5,CD44,DUSP	228 (16)
8	STAT1		transcription regulator	Activated	3.6	bias	0.00407	BAX,CASP8,CCL4,CCL5,CCR7,CDKN	245 (19)
9	IL-12 (complex)		complex	Activated	3.2	bias	8.38E-08	CCL4,CCL5,CCR7,CD44,CYTIP,DDB1	214 (18)
10	IL-13		cytokine	Activated	3.2	bias	0.0000187	ACADVL,ACSL1,ADAM8,BAX,BID,C	255 (21)
11	IL-15		cytokine	Activated	3.1	bias	1.85E-18	ALDOA,ATF4,CALM1 (includes others	270 (21)
12	EIF2AK2		kinase	Activated	3.1	bias	0.000343	ATF4,BAX,CEBPB,CEBPD,FOS,IFI27	205 (21)
13	NFKB2		transcription regulator	Activated	3.0	bias	0.00000891	AKR1B1,ANXA5,CCL5,CCR7,FCER2,	222 (17)
14	TCF7L2		transcription regulator	Activated	3.0	bias	0.00638	ACSL1,ADGRG1,ALOX5,CDKN1B,EI	220 (17)
15	IL-1A		cytokine	Activated	2.9	bias	0.00993	BAX,CCL4,CCL5,CD44,FAM89B,FOS	228 (18)
16	IL-27		cytokine	Activated	2.8	bias	0.000347	CCL4,CCL5,CD74,CEBPD,FOS,HLA-E	217 (18)
17	CXCL12		cytokine	Activated	2.7	bias	0.000000555	BAX,CCL5,CD3G,CD44,CD9,DAD1,F	237 (18)
18	PTGS2		enzyme	Activated	2.7	bias	0.000108	ANXA2,BAX,CCL4,CCL5,CCR7,CD44	161 (14)
19	CCL2		cytokine	Activated	2.7	bias	0.00737	CCL5,CEBPB,CEBPD,FOS,ITGA5,ITG	174 (17)
20	CD3		complex	Activated	2.6	bias	1.16E-15	ATF4,CALM1 (includes others),CASP8	243 (23)
21	NFKB1		transcription regulator	Activated	2.6	bias	0.000721	AKR1B1,ATP6AP2,BAX,BTG2,CCL4,	228 (17)
22	AKT1		kinase		2.6	bias	0.00698	BAX,CCL4,CCL5,CD74,CDKN1B,CEE	322 (21)
23	IL-18		cytokine	Activated	2.5	bias	0.0000075	CCL4,CCL5,CCR7,CD44,CXCL16,FCG	258 (19)
24	CD2		transmembrane receptor	Activated	2.4	bias	0.0000534	CCR7,CD44,CD8A,HLA-DMA,ITGAL	181 (17)
25	PIK3CA		kinase	Activated	2.4		0.000274	CD44,CDKN1B,CEBPB,CEBPD,ITGA	218 (18)
26	NFKBIA		transcription regulator	Activated	2.3	bias	0.00000121	BAX,BID,BTG2,CASP8,CCL5,CDC25I	258 (17)
27	IL-21		cytokine	Activated	2.2	bias	0.0000696	CCL4,CCL5,CCR7,CDKN1B,ID2,IL7R,	256 (19)
28	CCL3		cytokine	Activated	2.2	bias	0.00111	CCL4,FOS,ITGA5,ITGAM,ITGB1	144 (14)
29	CCR5		G-protein coupled receptor	Activated	2.1	bias	0.0000534	BAX,BID,CASP8,CCL4,CCL5,CD9,FO	237 (17)
30	MAPK8		kinase	Activated	2.1	bias	0.000487	CCL5,CDKN1B,CEBPB,CXCL16,DGA	263 (17)
31	IL-7		cytokine	Activated	2.1	bias	0.00105	BAX,CCL4,CD247,CD3D,CD3G,CD8A	198 (20)
32	CXCL3		cytokine		2.0	bias	0.00236	CCL4,CCL5,GRK2,ITGB1	169 (14)

Table 13B: IPA analysis of paired (miRNA - mRNA) gene expression of "upstream regulators" (down-regulated [25]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

	Genes	Expression	Molecule Type	Predicted	Activation	Flags	*P*-value	Target Molecules in data set	Mechanistic
#		Log Ratio		Activatin State	z-zone		overlap		Network
33	MAPK1		kinase		-0.9		0.00000558	ANPEP,CCL4,CCL5,CDKN1B,CPPED1	288 (22)
34	mir-10		microRNA	Inhibited	-3.6	bias	0.00000592	ALOX5,BAX,CD44,DGAT1,HTATIP2	177 (13)
35	PTPN6		phosphatase		-1.5	bias	0.0000209	BAX,CCR7,CD44,CDKN1B,ETS1,IRF1	215 (18)
36	mir-21		microRNA	Inhibited	-4.7	bias	0.0000391	ARF4,ARF6,BTG2,CCT8,CLU,COPG1	185 (12)
37	mir-148		microRNA		-1.8	bias	0.0000554	IKZF1,PKM,PTEN,RELA,ROCK1,RUI	
38	MIR-124		group		-1.9	bias	0.0000935	PTPRC,RELA,RHOG,ROCK1,SET,SPI	
39	mir-122		microRNA	Inhibited	-3.3	bias	0.000161	ALDOA,CCNG1,G6PD,NUDC,NUTF2	
40	miR-193a-3p (and other		mature microRNA		-2.0	bias	0.000305	ETS1,MCL1,RPS6KB2,SRSF2	117 (7)

41	miR-29b-3p (and other r		mature microRNA	Inhibited	-2.9	bias	0.000307	ARPC3,CDC42,CNOT8,FRAT2,HMG	
42	miR-155-5p (miRNAs w		mature microRNA	Inhibited	-3.7	bias	0.000312	CCL4,CEBPB,DNAJB1,ETS1,FCER2,I	
43	mir-155		microRNA	Inhibited	-2.9	bias	0.000469	ANXA2,CCL5,CEBPB,DNAJB1,FCER	228 (18)
44	Akt		group		-0.4	bias	0.00117	ATF4,BAX,BID,BTG2,CCR7,CD44,CI	253 (16)
45	BCL2		transporter		-0.5	bias	0.00275	BAX,CCNG1,CDKN1B,CTSH,FOS,IT	290 (21)
46	miR-125b-5p (and other		mature microRNA	Inhibited	-3.0	bias	0.00352	ALOX5,ANAPC16,CD44,DUS1L,ID2,I	
47	IL-10RA	3.613	transmembrane receptor		-0.9		0.00691	ACSL1,ADD3,ALOX5,BAX,CCL5,CCI	170 (15)
48	miR-16-5p (and other m		mature microRNA	Inhibited	-3.7	bias	0.0074	ANAPC16,CAPRIN1,GRB2,HMGA1,I	
49	mir-1		microRNA	Inhibited	-2.9	bias	0.0103	ANXA2,CAPRIN1,CEBPB,LASP1,PIC	
50	mir-210		microRNA	Inhibited	-2.7	bias	0.0115	DDOST,FCER2,IL16,KIAA0930,KLF6,	
51	mir-181		microRNA		-2.0	bias	0.0179	DUSP6,KLF6,MCL1,PRKCD,PTEN	
52	mir-193		microRNA		-2.0	bias	0.0194	CD151,MCL1,PTEN,RPS6KB2	
53	mir-15		microRNA		-1.6	bias	0.0216	CCL5,CDC42,HSPA1A/HS	
54	IL-1RN		cytokine	Inhibited	-3.0	bias	0.0252	CD44,IFI27,IL32,IRF1,IRF9,	
55	miR-291a-3p (and other		mature microRNA	Inhibited	-2.6	bias	0.0259	CD44,KLF13,PCGF5,PHC2,RELA,STK	
56	miR-199a-5p (and other		mature microRNA		-1.4	bias	0.0262	ETS1,IFI27,KLF2,MX2,MYL9,SET	
57	mir-8		microRNA		-0.6	bias	0.0263	BAX,CDC25B,CRTAP,ETS1,HDAC3,I	
58	miR-145-5p (and other r		mature microRNA	Inhibited	-2.2	bias	0.04	AHNAK,FLI1,LAMP2,MDM2,MYL9	
59	mir-29		microRNA	Inhibited	-2.4	bias	0.0437	ARPC3,CDC42,MCL1,PTEN,TBX21,T	

The "up-stream regulators", out of total 1550, only 32 (up-regulated) and 25 down-regulated covering all important genes associated with diabetes (Tables 13A, 13B).

Table 14A: IPA analysis of paired (mRNA-miRNA) of gene expression of "disease functions" (up-regulated [54]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Diseases or Functions Annotation	*P*-value	Predicted Activation State	Activation z-score	Molecules	Number of Molecules
1	Cell survival	2.05E-18	Increased	7.7	AGTRAP,ANXA5,APEX1,AT	150
2	Cell viability	1.93E-17	Increased	7.6	AGTRAP,ANXA5,APEX1,AT	142
3	Infection by RNA virus	1.18E-14	Increased	7.0	ALOX5,ANXA2,ARF1,ARF6,A	96
4	Cell movement	8.6E-22	Increased	7.0	ABI3,ADAM8,ADGRG1,AHN	216
5	Infection of cells	1.36E-12	Increased	7.0	ANXA2,ARF1,ARF6,ARPC1A	81
6	Migration of cells	2.8E-19	Increased	6.8	ABI3,ADAM8,ADGRG1,AHN	193
7	Migration of mononuclear leukocytes	3.27E-19	Increased	5.9	ALOX5,ANXA2,APBB1IP,CA	63
8	Cell movement of blood cells	1.73E-23	Increased	5.7	ADAM8,AKR1B1,ALOX5,AN	124
9	Leukocyte migration	6.79E-23	Increased	5.6	ADAM8,ALOX5,ANXA2,ANX	122
10	Cell movement of tumor cell lines	4.93E-13	Increased	5.6	AHNAK,ANPEP,ANXA2,ARF	102
11	Cellular homeostasis	1.7E-13	Increased	5.5	ACSL1,AGPAT2,AKR1B1,ALI	145
12	Lymphocyte migration	1.09E-17	Increased	5.5	ALOX5,APBB1IP,CALR,CCL4	58
13	Synthesis of lipid	3.88E-08	Increased	5.0	ACADVL,ACSL1,AGPAT1,AC	74
14	Binding of lymphatic system cells	6.39E-15	Increased	5.0	ANXA2,APBB1IP,CALR,CCL4	35
15	Adhesion of immune cells	8.2E-16	Increased	4.9	ANXA5,APBB1IP,CCL4,CCL5	54
16	Migration of tumor cell lines	2.03E-11	Increased	4.8	AHNAK,ANPEP,ANXA2,ARF	86

17	Invasion of cells	6.12E-15	Increased	4.7	ADAM8,AHNAK,ALOX5,AN	105
18	Binding of blood cells	3.86E-17	Increased	4.6	ANXA5,ANXA7,APBB1IP,CA	63
19	Adhesion of lymphatic system cells	5.07E-13	Increased	4.5	ANXA2,APBB1IP,CCL4,CCR7	27
20	Adhesion of blood cells	6.14E-15	Increased	4.5	ANXA5,ANXA7,APBB1IP,CC	55
21	Infection of epithelial cell lines	6.94E-09	Increased	4.4	BTG2,CDC42EP3,CTBP1,DAZ	33
22	Adhesion of lymphocytes	8.06E-13	Increased	4.4	APBB1IP,CCL4,CCR7,CD151,(	26
23	Cell movement of breast cancer cell line	5.89E-12	Increased	4.3	AHNAK,ANXA2,ARF1,ARF6,	47
24	T cell migration	2.11E-12	Increased	4.3	ALOX5,CALR,CCL4,CCL5,CC	41
25	Endocytosis	5.54E-14	Increased	4.3	ACTG1,ANK1,ANXA5,ARF1,	68
26	Formation of cytoskeleton	6.03E-10	Increased	4.2	AKAP9,ARF1,ARF6,ARHGEF	48
27	Aggregation of cells	5.37E-11	Increased	4.1	AKR1B1,ANXA7,CAPN1,CAS	43
28	Development of cytoplasm	9.96E-11	Increased	4.1	AKAP9,ANKRD13D,ARF1,AF	58
29	Cell movement of phagocytes	2.22E-16	Increased	4.1	ADAM8,ALOX5,ANXA2,BID	76
30	Invasion of tumor cell lines	7.23E-12	Increased	4.1	ADAM8,AHNAK,APEX1,ARF	83
31	Homeostasis of blood cells	8.33E-11	Increased	4.1	BAX,C5AR1,CASP8,CCL5,CC	65
32	Adhesion of tumor cell lines	2.95E-11	Increased	4.0	ADD3,ANXA2,CASP8,CCL4,C	43
33	Chemotaxis of blood cells	9.3E-14	Increased	4.0	ADAM8,ALOX5,C5AR1,CCL4	51
34	Chemotaxis of leukocytes	3.24E-13	Increased	4.0	ADAM8,ALOX5,C5AR1,CCL4	50
35	Cell movement of T lymphocytes	1.09E-09	Increased	4.0	CALR,CCL4,CCL5,CCR7,CD24	34
36	Proliferation of blood cells	1.5E-21	Increased	3.1	AHNAK,ANPEP,ARF6,BAX,C	110
37	Expression of RNA	3.59E-14	Increased	3.0	ABHD14B,AKNA,ANXA7,AR	187
38	Proliferation of immune cells	4.02E-22	Increased	2.9	AHNAK,ANPEP,ARF6,BAX,C	104
39	Cytotoxicity of lymphocytes	1.96E-08	Increased	2.9	CCL5,CD247,CD44,FCGR2A,F	23
40	Cell cycle progression	1.22E-10	Increased	2.9	AHNAK,AKAP9,ARPC1B,AR	98
41	Migration of carcinoma cell lines	0.00000052	Increased	2.8	ANPEP,ARL4C,BRMS1,CALR	27
42	Autophagy	0.00000113	Increased	2.8	ACSL1,ANXA7,ARF6,ATF4,B	45
43	Cell movement of hematopoietic proge	8.94E-09	Increased	2.8	CCL4,CCL5,DIAPH1,GNAI2,H	16
44	Cell death of immune cells	4.22E-16	Increased	2.4	ADAM8,ANPEP,BAX,BID,CA	81
45	Cell proliferation of T lymphocytes	5.71E-21	Increased	2.3	AHNAK,ARF6,BAX,C5AR1,C	86
46	Apoptosis of fibroblast cell lines	1.49E-11	Increased	2.2	ATP6AP2,BAX,BID,CASP8,C(	44
47	Insulin-dependent diabetes mellitus	0.000000228		1.2	ACSL1,AGPAT1,APOBR,CAM	45
48	Diabetes mellitus	0.000000478		0.5	ACSL1,AGPAT1,AGPAT2,AK	89
49	Apoptosis of leukocytes	1.84E-11		0.4	ADAM8,ANPEP,BAX,BID,CA	55
50	Apoptosis of T lymphocytes	3.38E-08		0.4	ADAM8,BAX,BID,CASP8,CC	34
51	Necrosis of prostate cancer cell lines	0.000000502		0.4	ALOX5,BAX,CALR,CASP8,CI	26
52	Expression of mRNA	0.0000006		0.3	BTG2,CALR,CAPRIN1,COPZ1	29
53	Apoptosis of hematopoietic cell lines	2.28E-11		0.1	BID,CABIN1,CASP8,CCL5,CD	32
54	Apoptosis of lymphoid cells	2.53E-09		0.1	ADAM8,BAX,BID,CAMLG,C	42

Table 14B: IPA analysis of paired (mRNA-miRNA) of gene expression of "disease functions" (down-regulated[18]) of NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Diseases or Functions Annotation	*P*-value	Predicted Activation State	Activation z-score	Molecules	Number of Molecules
55	Organismal death	1.33E-08	Decreased	-11.096	ACADVL,ACTG1,ADGRG1,A	178
56	Morbidity or mortality	7.52E-09	Decreased	-10.911	ACADVL,ACTG1,ADGRG1,A	181
57	Infection of mammalia	3.7E-11	Decreased	-3.911	AHNAK,AKNA,C5AR1,CASP	43
58	Hyperplasia of lymphoid organ	0.000000862	Decreased	-3.796	CASP8,CDKN1B,FLI1,IKZF1,I	15
59	Anemia	3.54E-13	Decreased	-3.599	ACADVL,ADD3,ALDOA,ANK	58
60	Epithelial neoplasm	1.5E-11	Decreased	-3.516	AAMP,ABHD14B,ABHD17A,	622
61	Tumorigenesis of tissue	2.82E-13	Decreased	-3.152	AAMP,ABHD14B,ABHD17A,	629
62	Non-hematological solid tumor	1.98E-13	Decreased	-3.13	AAMP,ABHD14B,ABHD17A,	636
63	Apoptosis	1.71E-26	Decreased	-3.02	ACSL1,ADAM8,AGPAT2,AK	249
64	Thrombocytopenia	0.000000111	Decreased	-3.009	BAX,CALR,CAMLG,CD44,CD	29
65	Non-melanoma solid tumor	1.47E-12	Decreased	-2.849	AAMP,ABHD14B,ABHD17A,	629
66	Carcinoma	1.54E-11	Decreased	-2.731	AAMP,ABHD14B,ABHD17A,	621
67	Nonhematologic malignant neoplasm	5.24E-13	Decreased	-2.641	AAMP,ABHD14B,ABHD17A,	633
68	Inflammation of organ	1.67E-10	Decreased	-2.512	ACADVL,AHNAK,ALDOA,A	122
69	Hemorrhagic disease	2.96E-08	Decreased	-2.393	BAX,CALR,CAMLG,CCL4,CC	34
70	Inflammation of body cavity	0.000000097	Decreased	-2.377	ALDOA,ALOX5,ANXA5,ANX	89
71	Quantity of interleukin	0.00000118	Decreased	-2.051	APEX1,C5AR1,CD44,CEBPB,C	19
72	Inflammation of absolute anatomical re	6.62E-08	Decreased	-2.044	ALDOA,ALOX5,ANXA5,ANX	101

The IPA analysis of paired mRNAs – miRNAs of gene expression of "diseases function, out of which a total of 500, only 54 up-regulated and 18 down-regulated involves with diabetes (Tables 14A, 14B). The most important up-regulated disease functions are cell survival, cell viability, infection by RNA virus, cell movement, infection of cells, migration of mononuclear leukocytes, cell movement of tumpr cell lines, synthesis of lipids, adhesion of immune cells, homeostasis of blood, cell proliferation of lymphocytes, and apoptosis of t lymphcytes. Whereas, down-regulation are organismal death, morbidity or mortality, infection of mammalian cells and anemia.

Table 15: IPA analysis of paired (mRNA-miRNA) of gene expression of "molecules in networks & top diseases & functions" (25 of each) after NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
1	ANXA2,CCNDBP1,CCT8,COL6A2,CRTA	46	31	[Cell Death and Survival, Infectious Diseases, RNA Post-Transcriptional Modification]
2	ACTG1,atypical protein kinase C,C4orf3,C/	44	30	[Cell Morphology, Cellular Assembly and Organization, Cellular Movement]
3	Ap2,Ap2 alpha,ASNA1,ATP6AP2,ATP6V(	42	29	[Cellular Compromise, Cellular Development, Inflammatory Response]
4	ABI3,B4GALT7,CAVIN2,CD99,Cdc42ep,(	42	29	[Gene Expression, RNA Damage and Repair, RNA Post-Transcriptional Modification]
5	AHNAK,CASC3,CDK4/6,CLN3,cytochrom	42	29	[Cancer, Protein Synthesis, RNA Damage and Repair]
6	26s Proteasome,ABHD17A,ANKRD13D,A	42	29	[Cell Cycle, Cellular Function and Maintenance, Post-Translational Modification]
7	60S ribosomal subunit,APC/APC2,CHTOP,(	39	28	[Cellular Assembly and Organization, Protein Synthesis, RNA Damage and Repair]
8	ALOX5,ANAPC15,ANAPC16,ANKRD44,	37	27	[Cell Cycle, Cell Morphology, Gene Expression]
9	ACADVL,CD3 group,CD3E,Complement,c	35	26	[Cell Signaling, Post-Translational Modification, Protein Synthesis]
10	AGTRAP,Alpha tubulin,ANK1,ASCL2,AU	31	24	[Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking]
11	ACTR10,Alp,BAX,BTBD2,C12orf75,CAP	31	24	[Cellular Compromise, Cellular Function and Maintenance, Neurological Disease]
12	APMAP,CHD3,CTBP1,DDB1,DMAC2,ES	31	24	[Developmental Disorder, Hereditary Disorder, Neurological Disease]

13	1-acylglycerol-3-phosphate O-acyltransferas	29	23	[Cellular Assembly and Organization, Energy Production, Nucleic Acid Metabolism]
14	ALDOA,ALT,CAPRIN1,DBI,DGAT1,DN	25	21	[Cardiovascular Disease, Cell Death and Survival, Connective Tissue Disorders]
15	ABHD14B,Ap1,APRT,ARL4C,ATF4,BRD	25	21	[Cellular Development, Cellular Growth and Proliferation, Embryonic Development]
16	20s proteasome,CD247,CD37,CD3D,CD3G	23	20	[Cancer, Hematological Disease, Immunological Disease]
17	AP5Z1,ARHGAP24,BCL9,C12orf75,C6orf	22	20	[Cell Death and Survival, Cellular Assembly and Organization, Nervous System Development and
18	ALPL,ARHGEF2,ATPase,C20orf27,Calcine	20	18	[DNA Replication, Recombination, and Repair, Nucleic Acid Metabolism, Small Molecule Bioch
19	ADAM8,BCR (complex),CCL4,CLEC7A,C	20	18	[Hematological System Development and Function, Lymphoid Tissue Structure and Development,
20	alcohol group acceptor phosphotransferase,A	20	18	[Cell Morphology, Cellular Assembly and Organization, Cellular Function and Maintenance]
21	CYBC1,CYTH1,CYTIP,Cytokeratin,ETS1,I	18	17	[Cell Death and Survival, Cell Morphology, Hematological System Development and Function]
22	ANXA5,C1q,CALR,CARS2,CAST,Collager	18	17	[Cell-To-Cell Signaling and Interaction, Cellular Compromise, Inflammatory Response]
23	AKNA,APP,ATRAID,BLOC1S1,BOLA1,C	18	17	[Cell-To-Cell Signaling and Interaction, Cellular Compromise, Inflammatory Response]
24	APMAP,ARL8A,C19orf38,CARD19,CHIP	18	17	[Cancer, Cell Morphology, Cellular Function and Maintenance]

The fold change in gene expression in paired mRNAs-miRNAs of top diseases and functions are (25) out of 500. The important one are cell death, infectious diseases, and

RNA post-transcriptional modification.

Table 16: IPA analysis of paired (mRNA-miRNA) of "canonical signaling pathways" (53) after NS-3 treated RNAs of people with type 2 diabetes mellitus.

#	Ingenuity Canonical Pathways	Exp-Log (*P*-value)	Ratio	z-score	Molecules
1	Integrin Signaling	16.4	0.2	4.1	ACTG1,ARF1,ARF4,ARF6,ARPC1A,ARPC1B,A
2	Actin Nucleation by ARP-WASP Complex	12.2	0.3	3.0	ARPC1A,ARPC1B,ARPC3,ARPC5,ARPC5L,CD
3	Cdc42 Signaling	10.7	0.2	3.1	ARPC1A,ARPC1B,ARPC3,ARPC5,ARPC5L,CD
4	EIF2 Signaling	8.5	0.1	1.9	ATF4,EIF1,EIF2A,EIF2S3,EIF3F,EIF3H,EIF3I,GI
5	CD28 Signaling in T Helper Cells	8.1	0.2	3.5	ARPC1A,ARPC1B,ARPC3,ARPC5,ARPC5L,CA
6	Protein Kinase A Signaling	7.7	0.1	0.0	ADD3,AKAP9,APEX1,ATF4,CALM1 (includes o
7	Role of NFAT in Regulation of the Immune Response	7.1	0.1	3.6	CABIN1,CALM1 (includes others),CD247,CD3D,
8	IL-8 Signaling	7.0	0.1	4.1	BAX,CDC42,EIF4EBP1,FOS,GNAI2,GNG10,GN
9	Mitochondrial Dysfunction	6.9	0.1	#NUM!	ATP5MC2,ATP5PB,CASP8,COX4I1,COX7A2L,
10	Production of Nitric Oxide and Reactive Oxygen Specie	6.8	0.1	2.1	CDC42,CLU,FOS,IRF1,LYZ,NCF4,PPP1CA,PPP
11	Thrombin Signaling	6.6	0.1	3.9	ARHGEF2,CAMK2G,CDC42,GNAI2,GNG10,GN
12	ERK/MAPK Signaling	6.6	0.1	1.1	ATF4,DUSP1,DUSP6,EIF4EBP1,ETS1,FOS,GRB
13	mTOR Signaling	6.6	0.1	2.7	CDC42,EIF3F,EIF3H,EIF3I,EIF4B,EIF4EBP1,PPI
14	Oxidative Phosphorylation	6.5	0.1	4.0	ATP5MC2,ATP5PB,COX4I1,COX7A2L,COX7B
15	Th1 and Th2 Activation Pathway	5.6	0.1	#NUM!	CD247,CD3D,CD3E,CD3G,CD8A,GRB2,HLA-B,
16	Type I Diabetes Mellitus Signaling	5.0	0.1	3.2	BID,CASP8,CD247,CD3D,CD3E,CD3G,HLA-B,H
17	Interferon Signaling	4.9	0.2	1.9	BAX,IFI35,IFITM2,IFITM3,IRF1,IRF9,PSMB8,I
18	T Cell Receptor Signaling	4.5	0.1	#NUM!	CALM1 (includes others),CD247,CD3D,CD3E,CD
19	Molecular Mechanisms of Cancer	4.2	0.1	#NUM!	ARHGEF2,BAX,BID,CAMK2G,CASP8,CDC25B
20	Protein Ubiquitination Pathway	4.2	0.1	#NUM!	DNAJA1,DNAJB1,DNAJC4,DNAJC8,HLA-B,HS
21	Insulin Receptor Signaling	3.8	0.1	1.2	EIF4EBP1,GRB2,GSK3A,INPP5D,PPP1CA,PPP1
22	B Cell Receptor Signaling	3.5	0.1	1.6	APBB1IP,ATF4,CALM1 (includes others),CAMK
23	Regulation of IL-2 Expression in Activated and Anergic	3.3	0.1	#NUM!	CALM1 (includes others),CD247,CD3D,CD3E,CD
24	IL-4 Signaling	2.8	0.1	#NUM!	FCER2,GRB2,HLA-B,HLA-DMA,HLA-DRA,HM
25	iNOS Signaling	2.6	0.1	#NUM!	CALM1 (includes others),FOS,HMGA1,IRF1,MY
26	IL-12 Signaling and Production in Macrophages	2.5	0.1	#NUM!	CEBPB,CLU,FOS,IRF1,LYZ,MYD88,PRKCD,PR

27	Apoptosis Signaling	2.4	0.1	1.0	BAX,BID,CAPN1,CASP8,MCL1,RAF1,RELA,R(
28	NF-κB Activation by Viruses	2.3	0.1	2.8	ITGA5,ITGAL,ITGB1,ITGB2,PRKCD,PRKCH,R
29	IL-17A Signaling in Fibroblasts	1.6	0.1	#NUM!	CEBPB,CEBPD,FOS,RELA
30	IL-1 Signaling	1.6	0.1	2.0	FOS,GNAI2,GNG10,GNG2,MYD88,PRKACA,R
31	Adipogenesis pathway	1.6	0.1	#NUM!	AGPAT2,CEBPB,CEBPD,CTBP1,HDAC1,HDA(
32	IL-10 Signaling	1.2	0.1	#NUM!	FCGR2A,FOS,IL10RA,IL4R,RELA
33	TNFR2 Signaling	1.2	0.1	#NUM!	FOS,RELA,TNFRSF1B
34	TREM1 Signaling	1.1	0.1	2.2	GRB2,ITGA5,ITGB1,MYD88,RELA
35	1,25-dihydroxyvitamin D3 Biosynthesis	1.0	0.3	#NUM!	POR
36	Oxidized GTP and dGTP Detoxification	1.0	0.3	#NUM!	RUVBL2
37	IL-6 Signaling	1.0	0.1	2.6	CEBPB,FOS,GRB2,MCL1,RAF1,RELA,TNFRSF
38	T Cell Exhaustion Signaling Pathway	1.0	0.1	1.3	FOS,HLA-B,HLA-DMA,HLA-DRA,IL10RA,IRF
39	JAK/Stat Signaling	0.9	0.1	2.2	CEBPB,FOS,GRB2,RAF1,RELA
40	tRNA Splicing	0.8	0.1	#NUM!	APEX1,TSEN34,TSEN54
41	p53 Signaling	0.7	0.1	#NUM!	BAX,CCNG1,HDAC1,MDM2,PTEN
42	NF-κB Signaling	0.7	0.0	1.4	CASP8,HDAC1,MYD88,PRKACA,RAF1,RELA,
43	Angiopoietin Signaling	0.7	0.1	1.0	DOK2,GRB2,RELA,TNIP1
44	SAPK/JNK Signaling	0.6	0.0	2.2	CDC42,GNG2,GRB2,HNRNPK,RAC2
45	eNOS Signaling	0.6	0.0	1.1	CALM1 (includes others),CASP8,HSPA1A/HSPA
46	IL-2 Signaling	0.5	0.0	#NUM!	FOS,GRB2,RAF1
47	tRNA Charging	0.5	0.1	#NUM!	CARS2,WARS
48	B Cell Activating Factor Signaling	0.4	0.0	#NUM!	FOS,RELA
49	Nitric Oxide Signaling in the Cardiovascular System	0.4	0.0	2.0	CALM1 (includes others),PRKACA,PRKCD,PRK
50	STAT3 Pathway	0.4	0.0	#NUM!	IL10RA,IL4R,IL7R,RAF1,TGFB1
51	Toll-like Receptor Signaling	0.4	0.0	#NUM!	FOS,MYD88,RELA
52	IL-17 Signaling	0.0	0.0	#NUM!	CEBPB,RELA
53	IL-15 Signaling	0.0	0.0	#NUM!	RAF1,RELA

The fold change gene expression of 53 out of 450 in canonical signaling pathways associated with T2DM are outlined in Table16.