JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Procyanidins Modulate MicroRNA Expression in Pancreatic Islets

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Supporting Information

ABSTRACT: Procyanidins modulate glucose metabolism, partly due to its effects on pancreas. Given the role of microRNAs (miRNAs) in the regulation of diabetes and the fact that flavonoids modulate miRNAs in other tissues, we hypothesized that procyanidins might target miRNAs in the pancreas. We investigated the miRNA expression profile in pancreatic islets isolated from rats treated with a daily dose of grape seed procyanidin extract (GSPE) (25 mg/kg of body weight) for 45 days. The miRWalk database identified putative target genes of these miRNAs. We found that GSPE altered significantly the expression of miR-1249, miR-483, miR-30c-1*, and miR-3544. In silico prediction studies suggested that ion transport and response to glucose are among the regulated pathways. As a conclusion, this is the first study showing that procyanidins can also exert their bioactivity on pancreatic islets by modifying the miRNA expression pattern.

KEYWORDS: *microRNA, procyanidins, pancreatic islets, apoptosis*

INTRODUCTION

MicroRNAs (miRNAs) are a family of small noncoding RNAs that post-transcriptionally regulate gene expression.[1] Each miRNA gene encodes a mature miRNA that is approximately 22 nucleotides in length. MiRNAs play predominantly inhibitory regulatory roles by binding to cis-elements in the 3′-untranslated region (UTR) of message-encoding RNAs.[2] This inhibition occurs by one of three mechanisms that are not mutually exclusive: target cleavage, repression of target translation, and message degradation in cytoplasmic P-bodies.[3] MiRNAs are important not only for normal organism development and physiology but also in cancer, heart disease, and inflammation.[2]

MiRNAs are also involved in diabetes. Studies in the Goto-Kakizaki rat, a model of spontaneous lean type 2 diabetes, showed differential expression of fifteen miRNAs in the skeletal muscle of these animals compared to Wistar control rats.[4] Herrera et al. recently showed that the expression pattern of five miRNAs in insulin target tissues is modified by hyperglycemia suggesting a role for these miRNAs in the pathophysiology of type 2 diabetes.[5] In addition, recent studies have demonstrated that miRNAs are required for pancreas development[6,7] and the regulation of glucose-stimulated insulin secretion.[8,9] The most studied miRNA molecule in the pancreas is miR-375. Studies have shown that miR-375 plays a role in pancreatic islet cell viability and function, and its knockdown or overexpression profoundly affects glucose metabolism.[2,10] Other miRNAs have been shown to regulate pancreas function including the regulation of insulin secretion by modulating the level of key components of exocytosis process and insulin biosynthesis (Table 1). Some miRNAs have also been related to β-cell apoptosis. Given the role of miRNAs in regulating processes that are important in disease states, including diabetes, targeting miRNAs with bioactive compounds may be a potential therapeutic strategy.

Procyanidins are phenolic compounds that are found in fruits, vegetables, chocolate, and beverages such as wine and

Table 1. MiRNA Action on β-Cells

microRNA	effects on β-cells	refs
miR-9	modify insulin secretion by modulating the level of key components of the exocytosis process	9, 47
miR-124a		33, 34
miR-96		33, 47
miR-375		2, 10
miR-130a		48
miR-200		48
miR-410		48
miR-33a		49
miR-30d	modify insulin biosynthesis	50, 51
miR-7		52
miR-15a		37
miR-19b		53
miR-24		54
miR-26		54
miR-148		54
miR-182		54
miR-34a	modify β-cell apoptosis	32
miR-146		32

Received: September 17, 2012
Revised: December 5, 2012
Accepted: December 6, 2012
Published: December 6, 2012

tea.[11] Procyanidins modulate glucose metabolism by modifying both glycemia and insulinemia (reviewed in ref 12). Procyanidins act as insulin-mimetic likely by targeting the liver and peripheral tissues. In glucose-altered metabolism, procyanidins prevent the induction of damage (fructose-feed models) and/or use alternative targets to exert their insulin-mimetic effects.[12] Our recent experiments also suggest that the pancreas is a target of procyanidins. We found that grape seed procyanidin extract (GSPE) affected insulinemia by modifying β-cell functionality and/or insulin degradation.[13] Some of these effects could be explained by its effect on membrane potential,[13] but we do not discard some other mechanistic explanation. Besides, grape seed procyanidins modulate apoptosis in Zucker Fatty rats[14] and cafeteria-fed rats.[15]

A few recent studies have shown that flavonoids can alter miRNA expression profiles. It has been demonstrated that GSPE modulates miRNA expression profiles in the hepatocyte cell line HepG2,[16] and repress miR-122 and miR-33 in the liver.[17] Coffee polyphenols increase miR-122 expression in Hepa 1−6 cells,[18] and quercetin downregulates the pro-inflammatory miR-155 in murine RAW264.7 macrophages.[19] Recently, Milenkovic described five hepatic miRNAs commonly modulated by nine different polyphenols.[20] Furthermore, flavonoids that have cancer preventative effects, such as genistein and epigallocatechingallate, also modify miRNA expression profiles in human uveal melanoma cells[21] and in HepG2 cells,[22] respectively. However, the majority of these studies were performed in vitro using cell lines but, to the best of our knowledge, there are no studies on the effects of flavonoids in vivo on the pancreas.

As miRNAs regulate pancreas function and apoptosis, we hypothesized that procyanidins effects in glucose homeostasis might also be mediated via modulating the pancreatic expression of miRNAs. Thus, the aim of this study was to investigate whether chronic procyanidin treatment modifies the expression of miRNAs in rat pancreatic islets and whether this is the mechanism that confers the effects of procyanidins on the pancreas.

MATERIALS AND METHODS

Chemicals. According to the manufacturer, GSPE (Les Dérives Résiniques et Terpéniques, Dax, France) contained monomeric (16.6%), dimeric (18.8%), trimeric (16.0%), tetrameric (9.3%), and oligomeric procyanidins (5 to 13 units, 35.7%) as well as phenolic acids (4.2%). For a detailed composition at mg compound/g of extract please check ref 23.

Animal Procedures. Female Wistar rats weighing 225−250 g were purchased from Charles River Laboratories (Barcelona, Spain) and housed in animal quarters at 22 °C with a 12 h light/12 h dark cycle. Treatment began after 1 week in quarantine. The animals were divided in two groups (10 animals per group): a control group and a group treated for 45 days with 25 mg GSPE per kg of body weight per day. The food (standard chow) was withdrawn at 8 a.m. every day, and at 8 p.m., the rats were treated with GSPE or vehicle (sweetened condensed milk diluted 1:6 with tap water, voluntary oral intake), after which the food was replaced. On the day of sacrifice, the animals, which had fasted overnight (fasting starts at 0.00 a.m.), were anesthetized at 9 a.m. using sodium pentobarbital (75 mg/kg of body weight), and then sacrificed by exsanguination. The blood was collected, and pancreatic islets were isolated from ten animals per group. One half of the pancreas from six rats per group were fixed overnight in 4% (w/v) formaldehyde (QCA, Amposta, Spain) and embedded in paraffin. This procedure was approved by the Experimental Animals Ethics Committee of the Universitat Rovira I Virgili (Permission number from the Government of Catalonia: 4250).

Islet Isolation. The islets were prepared by collagenase digestion as previously described.[13] Briefly, the rats were anesthetized, and the pancreas was infused with 7 mL of ice-cold collagenase P (Roche, Barcelona, Spain) solution (1 mg/mL) before its removal and incubation at 37 °C for 15 min. The islets were purified on a Histopaque gradient (Sigma-Aldrich, St. Louis, MO) and selected by hand until a population of pure islets was obtained.

miRNA Profile Analysis. The total RNA from freshly isolated islets was extracted with the Qiagen miRNeasy isolation kit (Qiagen, Barcelona, Spain) and stored at −80 °C. The quality of the total RNA was determined with the Agilent 2100 Bioanalyzer using the RNA 6000 Nano Kit according to the manufacturer's instructions. All samples have a ratio of 260/280 absorbance of approximately 2.0 and a ratio of 260/230 absorbance greater than 1.4. We pooled the samples from 5 animals per treatment, that is two samples from a pool of two animals and one sample, only from one animal. Then we analyzed three samples from each treatment with a Geniom Realtime Analyzer (GRTA, febit GmbH, Heidelberg, Germany) using the Geniom Biochip MPEA *Rattus norvegicus*. The probes were designed as the reverse complements of all of the major mature miRNAs and the mature sequences as published in the current Sanger miRBase release (version 16.0 September 2010 for *Rattus norvegicus*).[24] For each array, the RNA was suspended in febit's proprietary miRNA Hybridization Buffer (25 μL per array). Hybridization was performed automatically for 16 h at 42 °C using the GRTA. Next, the biochip was stringently washed. Following the labeling procedure, febit was applied to the microfluidic-based primer extension assay.[25] This assay utilizes the bound miRNAs as a primer for enzymatic elongation using labeled nucleotides. The elongation was performed with Klenow fragment and biotinylated nucleotides at 37 °C for 15 min. Finally, the Biochip was washed automatically. For maximum sensitivity, febit method used biotin and its detection with streptavidin-phycoerythrin (SAPE) in combination with febit's consecutive signal enhancement (CSE) procedure. The feature recognition (using Cy3 filter set) and signal calculation were performed automatically within milliseconds. The Geniom technology provides accurate detection of miRNA profiles. The microarray data were normalized by the variance stabilization normalization method,[26] and the statistics were analyzed with linear models as implemented in the limma *Bioconductor* package.[27] Fold changes with adjusted *p*-values less than 0.2 were considered significant.

Pathway Analysis and Prediction. The predicted target genes for rno-miRNAs that had statistically significant changes in expression after GSPE treatment were obtained from the online database miRWalk.[28] MiRWalk miRNA target prediction was performed by the match among eight established miRNA prediction programs on 3′UTRs (RNA22, miRanda, miRDB, TargetScan, RNAhybrid, PITA, PICTAR, and Diana-microT) with a *p*-value less than 0.05.

To determine the functions of the common predicted target genes, we used the DAVID (Database for Annotation, Visualization and Integrated Discovery).[29] This database allowed us to assign predicted target genes to functional groups based on molecular function, biological process, and specific pathways. The GO terms with a *p*-value less than 0.05 after adjustment using the Benjamini method were considered significantly enriched.

To gain further knowledge of the pathways modified by procyanidins through miRNA the list of the common predicted target genes was further analyzed using the network building tool, Ingenuity Pathway Analysis (IPA) (Ingenuity Systems, Inc.), which uses the Ingenuity Pathways Knowledge Base. Hypothetical networks of the predicted target genes and genes from the Ingenuity database were built using the de novo network-building algorithm. IPA calculates a significance score for each network, where score = $-\log_{10}(p\text{-value})$. This score specifies the probability that the assembly of a set of genes in a network could be generated randomly. A score of 3 indicates that there is a 1 in 1000 chance that the focus genes are arranged together in a network due to random chance. Therefore, networks with scores of 3 or higher have a 99.9% confidence of not being generated by random chance (Ingenuity Systems, Inc., n.d.).

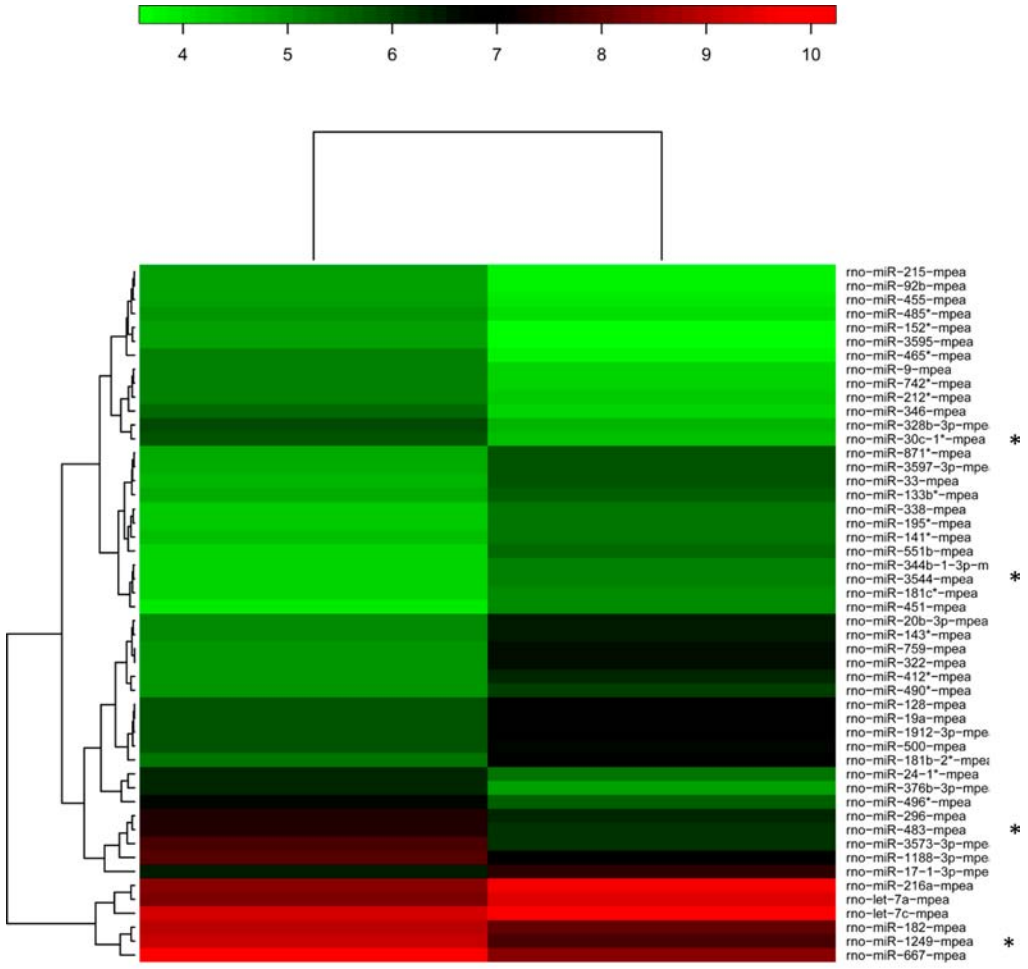

Figure 1. Differential expression of miRNAs in control rats (left column) vs GSPE-treated rats (right column) and a hierarchical clustering/heatmap of the 50 miRNA genes with the highest logFC values. The 4 miRNA genes with the most significantly altered expression are shown (*). The colorgram depicts high (red), average (black), and low (green) expression levels. Each row represents a miRNA, and each column represents a treatment.

Measurement of Apoptosis and Proliferation Marker Expression. For gene expression experiments, the total RNA from freshly isolated islets was extracted as described above (Qiagen miRNeasy isolation kit). RNA (0.5−1 μg) was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA), and cDNA was amplified for 40 cycles using a quantitative RT-PCR 7300 System (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions with TaqmanMaster Mix and the following Taqman probes (Applied Biosystems, Foster City, CA): Rn99999125_m1 for Bcl2, Rn01492401_m1 for Cyclin D2 (CCND2), Rn01451446_m1 for MKI67 and Rn00667869_m1 for β-actin as the reference gene. The relative mRNA expression levels were calculated using the $\Delta\Delta$Ct method.

Immunohistochemical Analysis of Apoptosis and Pancreatic Islet Mass. Blocks of paraffin-embedded pancreases were serially sectioned (6 μm) and mounted on slides. One section of every 40 was deparaffinized, rehydrated and analyzed for apoptosis. Apoptotic cells were detected with the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore, Billerica, MA) as previously described.[30]

Pancreas sections were then immunostained for insulin expression. First, they were blocked for 50 min at room temperature with goat serum (Vector Laboratories, Burlingame, CA) diluted 1/10 with Tris buffer and then incubated overnight at 4 °C with guinea pig anti-insulin antibody (MP Biomedicals, Illkirch, France) diluted 1/300 with Tris buffer. After washing, the samples were incubated for 1 h at room temperature with an alkaline phosphatase-conjugated polyclonal rabbit antiguinea pig antibody (Abcam, Cambridge, UK) diluted 1/200 with Tris buffer and then stained using the Alkaline Phosphatase Substrate kit I (Vector Laboratories, Burlingame, CA). After staining, the sections were dehydrated and mounted in Eukitt (Labonord, Templemars, France). β-cells were stained in red, and apoptotic cells had dark nuclei.

Analysis of the sections was performed with an Olympus BX40 microscope in conjunction with a video camera connected to a computer and *Histolab* software v. 7.2.7 (Microvision Instruments, Evry, France). The number of apoptotic cells, the surface area of the islets and the total pancreatic surface area were quantified in each stained section. The rate of apoptosis is expressed as the number of apoptotic cells/total pancreas surface area (μm^2) multiplied by 10^7, and β-cell mass was determined as the percentage of the ratio between the β-cell surface and the total pancreatic surface area.

Calculations and Statistical Analysis. The results are expressed as the mean ±SEM. The effects were assessed using Student's *t*-test. All calculations were performed with SPSS software.

RESULTS

Global miRNA Expression Profiling in Islets of GSPE-Treated Rats. To analyze whether GSPE alters the miRNA expression profile in the pancreas, we treated rats with a daily dose of GSPE for 45 days. Total RNA was obtained from freshly isolated islets, and the expression of 680 miRNAs was analyzed. In Figure 1, the 50 miRNAs with the highest fold

changes are shown. We found 4 miRNAs with significantly different expression after GSPE treatment (Table 2): miR-1249, miR-483, miR-30c-1*, and miR-3544. All of these were downregulated except for miR-3544, which was upregulated by GSPE treatment.

Table 2. Differentially Expressed MiRNAs in Pancreatic Islets Isolated from Rats Treated Chronically with a Daily Dose of Grape Seed Procyanidin Extract

miRNA	chromosome localization	fold change	*p*-value
upregulated			
rno-miR-3544	6q32	1.40	0.0010
downregulated			
rno-miR-1249	7q34	−1.72	0.0001
rno-miR-483	1q41	−1.39	0.0010
rno-miR-30c-1*	5q36	−1.36	0.0009

Integrative Analysis of miRNA Target Genes and Pathways. To investigate the function of these differentially expressed miRNAs, we analyzed their putative target genes using the miRWalk database.[28] MiR-1249 and miR-3544 do not have any predicted targets. MiR-483 has 1592 predicted targets, and miR-30c-1* has 2442 predicted targets.

To further define the effects of GSPE in the pancreas that are mediated by miRNAs, we explored whether the miRNAs modified by GSPE have common targets. We found that miR-483 and miR-30c-1* have 599 common targets. We next performed an in silico prediction to determine the functions of these predicted target genes using the ontology classification of genes based on gene annotation and summary information available through DAVID. This approach provides insights on which biological processes are modified by altered miRNA expression after GSPE treatment. We restricted the analysis to common predicted targets of miR-483 and miR-30c-1* (the other two miRNAs had no predicted targets) because it has been shown that the effects of binding multiple miRNA complexes to the 3′-UTR are cooperative; therefore, effects greater than those mediated by a single miRNA can be exerted.[31] As we are using predicted targets, we focused on the common targets that could suggest signaling pathways that are likely to be affected.

The significantly enriched Gene Ontology (GO) terms in the common predicted miRNA target genes are listed and classified in Table 3 according to the biological processes in which they are involved. The detailed list of predicted targets is provided as Table S1. The results indicate that most of the common miR-483 and miR-30c-1* target genes are involved in ion transport, response to stimulus such as hormones and organic substances, neuron differentiation and development, and transmission of nerve impulses. Moreover, the analysis of the GO cellular component category of the significantly enriched GO terms indicates that the majority of the common target genes are located on the plasma membrane, which could be predicted by their function.

To gain further knowledge we imported the common predicted miRNA targets into the *Ingenuity Pathway Analysis* software (parts A−D of Figure 2). We selected the best scored pathways defined by the program, that is those with a score higher than 30, to identify the most suitable pathways regulated by miRNA in pancreatic islets. As indicated by GO analysis, most of proteins corresponding to predicted genes (the gray one's) were in plasma membrane. Part A of Figure 2 showed that most of them interact to AKT. In part B of Figure 2, the central focus of all the interactions was ERK 1/2; in part C of Figure 2, the central point was p38, and part D of Figure 2 showed a less clear unique central target, but it reinforces ERK target.

GSPE Does Not Affect Apoptosis in Islets of Healthy Rats. MiRNA modify β-cell apoptosis[32] and we know that, procyanidins can modulate apoptosis markers in Zucker Fatty rats[14] and cafeteria-fed rats.[15] Since we are working on healthy animals, we checked if there was some apoptotic effect due to GSPE treatment. Immunohistochemical analysis did not reveal apoptosis in islets, and apoptosis in the exocrine pancreas was not altered by treatment with GSPE (part A of Figure 3). The β-cell mass was not altered by treatment with GSPE (part B of Figure 3). We also analyzed the expression of the antiapoptotic marker Bcl2 and the proliferation markers Cyclin D2 and MKI67. The islets of the rats treated with GSPE showed a trend of ($p < 0.1$) decreased expression of Bcl2 (0.75 ± 0.09 vs 1.02 ± 0.10), while MKI67 expression did not change (0.71 ± 0.22 vs 1.02 ± 0.11). However, Cyclin D2 was significantly downregulated by treatment with GSPE (0.77 ± 0.05 vs 1.02 ± 0.10) ($p < 0.05$).

DISCUSSION

In this study we show for the first time that grape seed procyanidins modulate miRNA expression in pancreas: it down-regulated miR-1249, miR-483, miR-30c-1*, and up-regulated miR-3544. Most of previous studies, regarding regulation of miRNAs by flavonoids, in different cell lines, did not identify any of these miRNAs as regulated by flavonoids.[16,19,21,22] However recently, Milenkovic, working on mice, showed five miRNAs (miR-291b-5p, miR-296−5p, miR-30c-1*, miR-467b*, and miR-374*) being commonly modulated by nine different polyphenols in the diet.[20] Despite that the work of Milenkovic was centered on liver tissue, they also found one miRNA common to our results, miR-30c-1*. This reinforces that it is a target for flavonoids. Besides, another of the four miRNAs that we found, miR-483, was also identified by Milenkovic as a target of mice that ingested narangin or curcumin.

Previous studies have reported that miRNAs play a role in the regulation of insulin secretion in rodent pancreatic β-cell lines (MIN6, INS-1E, and MIN6 B1 cells). Specifically, miR-375 has been well characterized in this function, but more recent studies have also suggested a role for miR-9, miR-124a, and miR-96, among others.[8,9,33,34] All of these miRNAs were present in our biochip, but GSPE did not alter their expression. Actually, of the nine flavonoids tested in the study of Milenkovic, only quercetin, which is not found in our extract, modulated miR-375. Instead, GSPE modulated the expression of other miRNAs for which, to our knowledge, a role in the pancreas has not been previously described. There is very little available information on the roles of these miRNAs. Only miR-483, which is a malignancy marker in adrenocortical tumors in humans,[35] and miR-30c-1*, which is associated with the recurrence of nonsmall cell lung cancer following surgical resection in humans, have been studied.[36] To overcome this limitation we used bioinformatics tools to elucidate the main β-cell functions modulated by these miRNAs.

MiRWalk database predicted 599 common targets of two of the miRNAs obtained. These targets were classified according to GO classification. Our results showed that the majority of the common target genes of the GSPE-modulated miRNAs are

Table 3. Significantly Enriched GO Terms in the Predicted Common Target Genes of MiR-483 and MiR-30c-1*

Biological process							GO term	Genes in pathway
Biological regulation	Regulation of biological process	Regulation of anatomical structure morphogenesis	Regulation of cell morphogenesis				0022604	14
		Regulation of cellular process						
		Regulation of cellular component organization						
Cellular process	Cellular component movement	Cell motility	Cell migration				0016477	19
	Cellular developmental process	Cell differentiation	Neuron differentiation				0030182	30
Developmental process	Anatomical structure development	Cell development	Neuron development				0048666	24
Localization	Establishment of localization	Transport	Ion transport				0006811	36
				Cation transport			0006812	28
					Metal ion transport		0030001	27
						Sodium ion transport	0006814	14
					Monovalent inorganic cation transport		0015672	22
						Sodium ion transport	0006814	14
			Neurotransmitter transport				0006836	14
Locomotion		Cell motility	Cell migration				0016477	19
Multicellular organismal process	System process	Neurological system process	Transmission of nerve impulse				0019226	20
Signalling	Multicellular organismal signalling							
Response to stimulus	Response to chemical stimulus	Response to organic substance					0010033	51
			Response to hormone stimulus				0009725	32
			Response to carbohydrate stimulus				0009743	12
				Response to monosaccharide stimulus			0034284	11
					Response to hexose stimulus		0009746	11
						Response to glucose stimulus	0009749	10
		Response to oxygen levels					0070482	16
	Response to endogenous stimulus						0009719	34
		Response to hormone stimulus					0009725	32

located on the plasma membrane. In this regard, our previous studies in pancreatic INS-1E cells have suggested that GSPE functions by altering membrane potential.[13] Indeed, we had previously shown that the experimental design used in the present study led to altered glucose-stimulated insulin secretion in the islets isolated from GSPE-treated rats.[13] This effect could be partially mediated by the modification of cell and mitochondrial membrane potentials by GSPE, which has been observed in the INS-1E cells. The role of other miRNAs in the regulation of pancreas function by modulating membrane proteins has previously been demonstrated. For example, miR-15a inhibits the expression of uncoupling protein-2,[37] a mitochondrial inner membrane uncoupler that modifies mitochondrial membrane permeability. Additionally, the expression levels of the plasma membrane monocarboxylaten transporter-1 are decreased in pancreatic β cells at least in part

dx.doi.org/10.1021/jf303972f | J. Agric. Food Chem. 2013, 61, 355−363

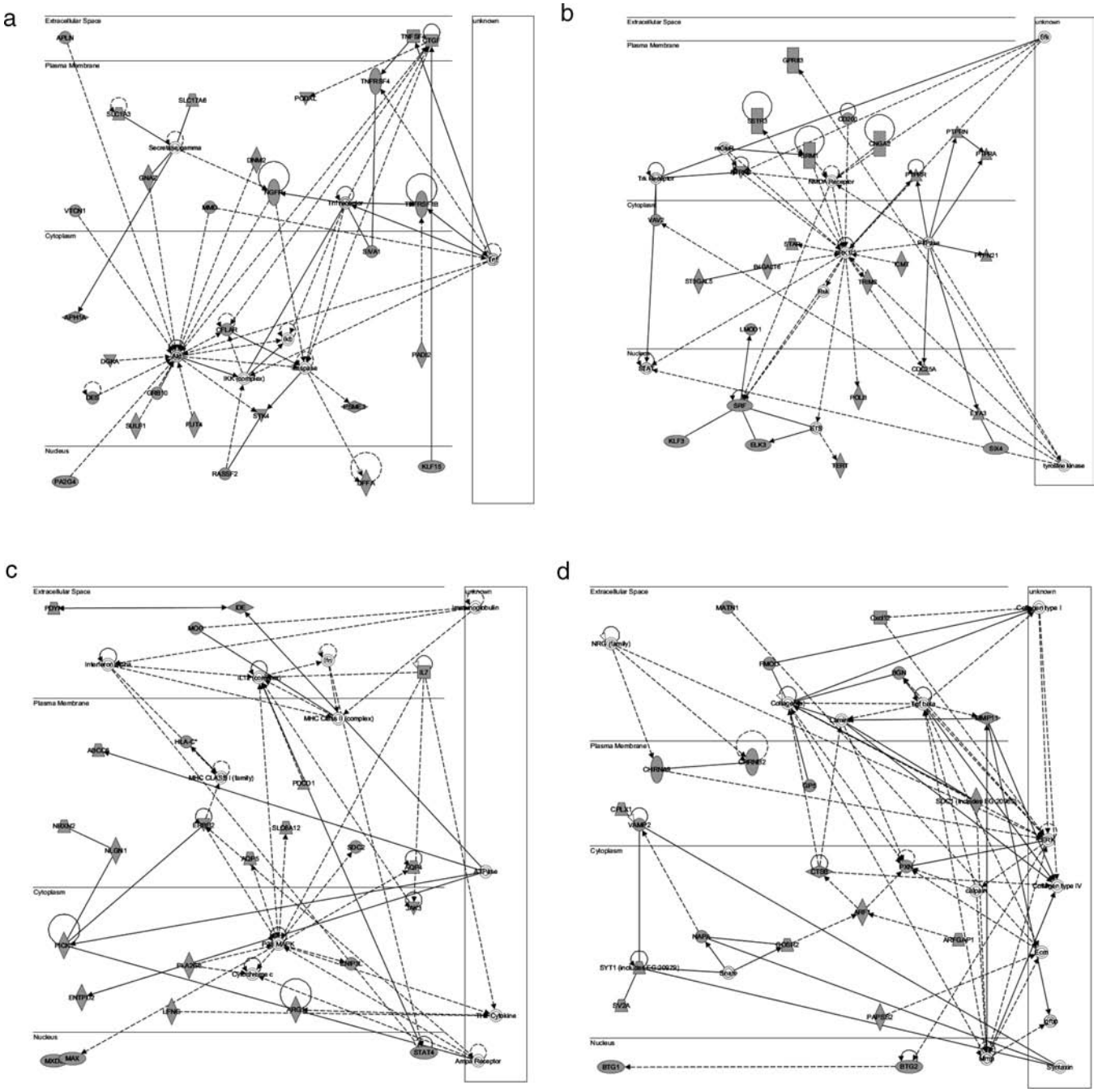

Figure 2. Hypothetical networks associated with the predicted target genes of the significantly altered miRNAs miR-483 and miR-30c-1* in the islets of the GSPE-treated rats generated by *Ingenuity Pathway Analysis* software. The best scored pathways were selected (a score higher than 30), A–D. Genes are represented as nodes with different shapes that represent different functional type of proteins. The predicted target genes are shown in gray and the genes depicted in white are genes from the Ingenuity database. The relationship between proteins is represented as a line, and the arrowheads indicate the direction of the interaction. A code description is included.

by miRNAs (miR-29a, miR-29b, and miR-124), thereby affecting insulin release.[38] Therefore, the effects of GSPE on the function of islets could in part be due to the effects of GSPE on the expression of miRNAs, which would contribute to changes in the cell and mitochondrial membrane permeability by varying the expression of ion transport proteins. To gain further knowledge on these possibilities, we charged the 599 predicted genes in *Ingenuity System Pathway Analysis*. This approach depicted networks modulated by these miRNA with some very clear nodes. These nodes correspond to proteins that, in previous studies from our research group, have been showed as modulated by procyanidins. AKT in adipocytes,[39] P38 and ERK in human monocytes.[40,41]

The amount of insulin production, that is the central function of β-cell, is highly dependent on the right synthesis and secretion of insulin but also on the amount healthy β-cells. Our results support that procyanidins, through miRNA, could alter insulin secretion. But most of the published studies on flavonoids and miRNAs have reported on the activity of these phenolic compounds in cancer, in which they regulated apoptosis and proliferation.[21,22,42−46] Besides, GSPE modulates apoptosis markers in genetically obese rats and rats fed a

dx.doi.org/10.1021/jf303972f | J. Agric. Food Chem. 2013, 61, 355−363

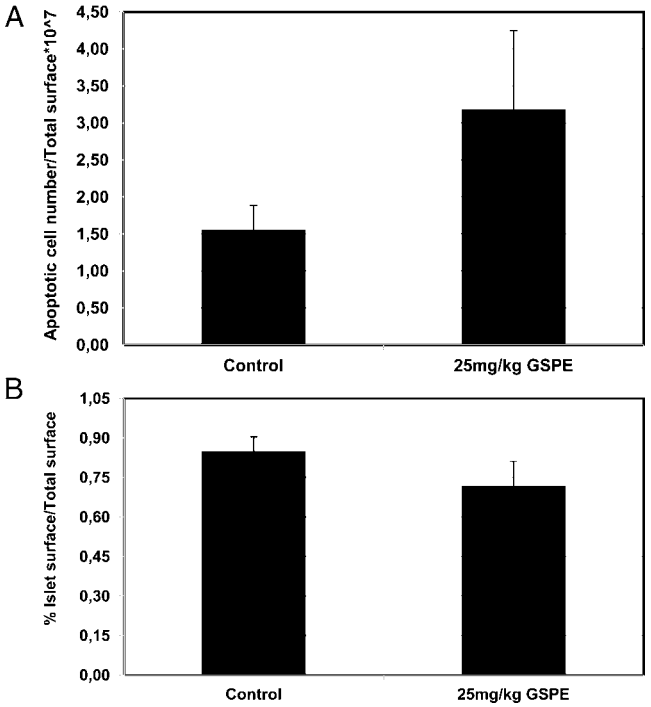

Figure 3. (A) Apoptosis in the exocrine pancreas measured by TUNEL assay. The rate of apoptosis is expressed as the number of apoptotic cells/total section surface area (μm^2) multiplied by 10^7. Data shown are the mean ± SEM from 3 samples per each group, and 5−6 sections were analyzed for each sample. (B) β-cell mass measured by insulin staining. The results were determined as the percentage of the ratio between the β-cell surface and the total pancreatic surface area. Data shown are the mean ± SEM from 4 samples per each group, and 4−10 sections were analyzed for each sample.

cafeteria diet.[15] As above-mentioned, we had restricted our bioinformatics analysis to the common predicted targets of the differentially expressed miRNAs, although this could leave out of the analysis other pathways modified by one of the miRNAs. Thus, given the evidence that flavonoids affect miRNAs involved in apoptotic processes, we also evaluated the possibility that the beta cell mass and apoptosis was affected by the current treatment. Our results showed that GSPE did not modulate the islet content or apoptosis in the pancreas. This reinforces the bioinformatics analysis that did not show apoptosis pathways controlled by the miRNAs.

In conclusion, we show that chronic GSPE treatment in rats modulates the miRNA expression profile in pancreatic islets, downregulating the expression of miR-1249, miR-30c-1*, and miR-483 and upregulating miR-3544. The limited amount of information available on these miRNAs makes it difficult to describe the consequences of their modulation by GSPE. Our in silico prediction studies combined with observations in previous cell culture studies suggest that ion transport and responses to glucose might be among the pathways affected. Thus, we have described a new mechanism of the effects of procyanidins on the pancreas.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed list of predicted targets. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This study was supported by a grant (AGL2008-01310) from the Spanish government. A. Castell-Auví is the recipient of an FPU fellowship from the Ministerio de Educación of the Spanish government. L. Cedó is the recipient of an FPI fellowship from Generalitat de Catalunya, and V. Pallarès is the recipient of a fellowship from Universitat Rovira i Virgili.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Joan Josep Sirvent (BioBanc Hospital Joan XXIII, Tarragona, Spain) for assistance with the pancreas sample preparation for immunohistochemistry. We thank Laura Guasch (Nutrigenomics Group, Universitat Rovira i Virgili, Tarragona, Spain) for assistance with data processing.

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