Gene 539 (2014) 213–223

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

MicroRNA expression profiling and functional annotation analysis of their targets in patients with type 1 diabetes mellitus

Paula Takahashi [a], Danilo J. Xavier [a], Adriane F. Evangelista [a,1], Fernanda S. Manoel-Caetano [a,b], Claudia Macedo [a,2], Cristhianna V.A. Collares [a,c], Maria C. Foss-Freitas [d], Milton C. Foss [d], Diane M. Rassi [c], Eduardo A. Donadi [a,c], Geraldo A. Passos [a,e], Elza T. Sakamoto-Hojo [a,b,*]

[a] Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo — USP, Av. Bandeirantes 3900, Monte Alegre 14049-900, Ribeirão Preto, SP, Brazil
[b] Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo — USP, Av. Bandeirantes 3900, Monte Alegre 14040-901, Ribeirão Preto, SP, Brazil
[c] Division of Clinical Immunology, Department of Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo — USP, Av. Bandeirantes 3900, Monte Alegre 14048-900, Ribeirão Preto, SP, Brazil
[d] Department of Internal Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo — USP, Av. Bandeirantes 3900, Monte Alegre 14048-900, Ribeirão Preto, SP, Brazil
[e] Disciplines of Genetics and Molecular Biology, Department of Morphology, Faculty of Dentistry of Ribeirão Preto, University of São Paulo — USP, Av. Do Café s/n, Monte Alegre 14040-904, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 2 September 2013
Received in revised form 18 December 2013
Accepted 29 January 2014
Available online 11 February 2014

Keywords:
Type 1 diabetes mellitus
microRNA
Microarray
Expression profile
T1DM candidate genes
Functional annotation analysis

ABSTRACT

Type 1 diabetes mellitus (T1DM) results from an autoimmune attack against the insulin-producing pancreatic β-cells, leading to elimination of insulin production. The exact cause of this disorder is still unclear. Although the differential expression of microRNAs (miRNAs), small non-coding RNAs that control gene expression in a post-transcriptional manner, has been identified in many diseases, including T1DM, only scarce information exists concerning miRNA expression profile in T1DM. Thus, we employed the microarray technology to examine the miRNA expression profiles displayed by peripheral blood mononuclear cells (PBMCs) from T1DM patients compared with healthy subjects. Total RNA extracted from PBMCs from 11 T1DM patients and nine healthy subjects was hybridized onto Agilent human miRNA microarray slides (V3), 8x15K, and expression data were analyzed on R statistical environment. After applying the rank products statistical test, the receiver-operating characteristic (ROC) curves were generated and the areas under the ROC curves (AUC) were calculated. To examine the functions of the differentially expressed (p-value < 0.01, percentage of false-positives < 0.05) miRNAs that passed the AUC cutoff value ≥ 0.90, the database miRWalk was used to predict their potential targets, which were afterwards submitted to the functional annotation tool provided by the Database for Annotation, Visualization, and Integrated Discovery (DAVID), version 6.7, using annotations from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. We found 57 probes, corresponding to 44 different miRNAs (35 up-regulated and 9 down-regulated), that were differentially expressed in T1DM and passed the AUC threshold of 0.90. The hierarchical clustering analysis indicated the discriminatory power of those miRNAs, since they were able to clearly distinguish T1DM patients from healthy individuals. Target prediction indicated that 47 candidate genes for T1DM are potentially regulated by the differentially expressed miRNAs. After performing functional annotation analysis of the predicted targets, we observed 22 and 12 annotated KEGG pathways for the induced and repressed miRNAs, respectively. Interestingly, many pathways were enriched for the targets of both up- and down-regulated miRNAs and the majority of those pathways have been previously associated with T1DM, including many cancer-related pathways. In conclusion, our study indicated miRNAs that may be potential biomarkers of T1DM as well as provided new insights into the molecular mechanisms involved in this disorder.
© 2014 Elsevier B.V. All rights reserved.

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; AGO1, argonaute-1; ALS, amyotrophic lateral sclerosis; AUC, area under the curve; CCL2, C–C motif chemokine 2; CCL3, C–C motif chemokine 3; CCL4, C–C motif chemokine 4; CDS, coding sequence; CTLA4, cytotoxic T-lymphocyte-associated protein 4; CXCL10, C–X–C motif chemokine 10; DAVID, database for annotation, visualization, and integrated discovery; DMSO, dimethylsulfoxide; F-actin, filamentous actin; FAK, focal adhesion kinase; GnRH, gonadotropin-releasing hormone; IAC, inter-array correlation; IL2RA, interleukin 2 receptor alpha; INS, insulin; KEGG, Kyoto encyclopedia of genes and genomes; LD, linkage disequilibrium; MAPK, mitogen-activated protein kinase; miRNAs, microRNAs; NOD, nonobese diabetic; NPH, neutral protamine Hagedorn; PBMCs, peripheral blood mononuclear cells; PFP, percentage of false-positives; PTPN22, protein tyrosine phosphatase, non-receptor type 22; RIN, RNA integrity number; ROBO1, roundabout, axon guidance receptor, homologue 1 (*Drosophila*); ROC, receiver-operating characteristic; SDF-1, stromal cell-derived factor-1; SLIT2, slit homologue 2; T1DM, type 1 diabetes mellitus; TGF-β, transforming growth factor-beta; *UTR*, untranslated region(s).

* Corresponding author at: Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo — USP, Av. Bandeirantes 3900, Monte Alegre 14040-901, Ribeirão Preto, Brazil. Tel.: +55 16 3602 3827; fax: +55 16 3602 0222.
E-mail address: etshojo@usp.br (E.T. Sakamoto-Hojo).
[1] Present address: Molecular Oncology Research Center, Barretos Cancer Hospital. R. Antenor Duarte Villela 1331, Paulo Prata 14784-400, Barretos, SP, Brazil.
[2] Present address: Department of Dental Materials and Prosthodontics, Faculty of Dentistry of Ribeirão Preto, University of São Paulo — USP, Av. Do Café s/n, Monte Alegre 14040-904, Ribeirão Preto, SP, Brazil.

http://dx.doi.org/10.1016/j.gene.2014.01.075
0378-1119/© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Type 1 diabetes mellitus (T1DM) is one of the major subtypes of diabetes mellitus, which is a group of chronic metabolic diseases that arises from a deficiency in insulin secretion and/or action, leading to chronic high blood glucose levels (hyperglycemia) (ADA, 2013). Chronic hyperglycemia, in turn, has been implicated in long-term complications involving a variety of organs, including kidneys, eyes, heart, nerves, and blood vessels (ADA, 2013).

T1DM is a polygenic disorder triggered by environmental factors that results from a T cell-mediated autoimmune assault against the insulin-producing β-cells localized in the pancreatic islets of Langerhans. As a consequence of this autoimmune attack, a decrease and eventually halt of insulin synthesis occur (ADA, 2013; van Belle et al., 2011). Thus, the conventional treatment for T1DM patients is daily exogenous insulin administration (van Belle et al., 2011). Even though this disorder generally occurs early in life, it can arise at any age (ADA, 2013; van Belle et al., 2011). Moreover, it is estimated that this type of diabetes affects 5–10% of all diabetic patients (ADA, 2013), with approximately 78,000 children worldwide developing T1DM every year (http://www.idf.org/diabetesatlas/5e/the-global-burden) (IDF, 2012).

Tiny RNA molecules, called microRNAs (miRNAs), are associated with several biological processes (such as development, differentiation, apoptosis, and proliferation) (Bartel, 2004), and interestingly, their differential expression has been detected in many disorders, such as different types of cancer (Dong et al., 2013; Jamieson et al., 2012; Lin et al., 2013; Martin et al., 2013; Zhang et al., 2013), stroke (Tan et al., 2013), type 2 diabetes (Balasubramanyam et al., 2011; Karolina et al., 2011), as well as cardiovascular (Bostjancic et al., 2010; Danowski et al., 2013), neurological (Minones-Moyano et al., 2011; Wong et al., 2013), and autoimmune diseases, including type 1 diabetes and associated nephropathy (Argyropoulos et al., 2013; Hezova et al., 2010; Liu et al., 2012; Nielsen et al., 2012; Pauley et al., 2008; Qin et al., 2013; Ridolfi et al., 2013; Salas-Perez et al., 2013; Sebastiani et al., 2011).

MiRNAs are endogenously expressed, evolutionarily conserved, small single-stranded non-coding RNAs of approximately 22 nucleotides in length that fine-tune gene expression (Bartel, 2004). In animals, miRNAs control gene expression in a post-transcriptional manner generally by partially base-pairing to specific sites located in the 3′ untranslated regions (*UTR*) of their target mRNAs, triggering destabilization and degradation and/or translational inhibition of the latter (Bartel, 2009; Krol et al., 2010; Lee et al., 1993; Lim et al., 2005; Wightman et al., 1993). Estimates revealed that approximately 50% of all mammalian protein-coding genes are under their post-transcriptional control (Krol et al., 2010). Since their discovery in the nematode *Caenorhabditis elegans* in 1993 (Lee et al., 1993; Wightman et al., 1993), thousands of miRNAs have been identified, and to date, more than 2000 mature miRNAs have been described in humans (miRBase release 19, August 2012) (Griffiths-Jones, 2004; Griffiths-Jones et al., 2006, 2008; Kozomara and Griffiths-Jones, 2011).

As aforementioned, there is evidence that miRNAs are involved in many pathological conditions; in fact, it has been suggested that miRNAs may represent potential biomarkers for the early detection as well as therapeutic targets for the treatment of diabetes (Mao et al., 2013). Nevertheless, all the factors that contribute to the development and/or pathogenesis of T1DM have not been completely elucidated and only limited knowledge exists regarding the expression of these small regulatory molecules in T1DM relative to healthy subjects (Hezova et al., 2010; Nielsen et al., 2012; Salas-Perez et al., 2013). Hence, in the present study, we employed the microarray technology, a powerful tool that allows the investigation of hundreds of miRNAs simultaneously, to investigate the miRNA expression profile exhibited by peripheral blood mononuclear cells (PBMCs) from T1DM patients compared with healthy subjects. By applying strict filtering criteria (p-value < 0.01, percentage of false-positives < 0.05, and area under the receiver-operating characteristic (ROC) curve (AUC) ≥ 0.90), we identified a set of potential miRNA markers (with several being reported for the first time in the present work), which were able to clearly stratify the two groups (T1DM patients and non-diabetic subjects). In addition, target prediction of those miRNAs indicated several candidate genes for T1DM. Furthermore, many downstream pathways are possibly regulated by those miRNAs, shedding light into the molecular mechanisms implicated in T1DM.

2. Material and methods

2.1. Study subjects

A total of 11 patients presenting type 1 diabetes mellitus (3 women and 8 men, mean age = 23.5 ± 3.9 years, ranging from 18 to 30), recruited while undergoing regular follow-up at the Outpatient Endocrinology of the Clinical Hospital — Faculty of Medicine of Ribeirão Preto, University of São Paulo (FMRP–USP), Brazil, and nine healthy subjects (control group) (5 women and 4 men, mean age = 25.1 ± 3.2 years, ranging from 20 to 29) participated in the present study. The main characteristics of all participants are described in Table 1. All patients were receiving treatment with human insulin and those presenting recent episodes of ketoacidosis and late diabetic complications, such as consolidated nephropathy, proliferative retinopathy, diabetic foot syndrome, autonomic neuropathy, and cardiovascular diseases, were excluded from the present study. Regarding the control group, individuals who were alcoholics, smokers, overweighed/obese, presented family history of diabetes, infections, hypertension, or long-term medication use were also excluded. The study protocol was approved by the local Ethics Committee of the Clinical Hospital — FMRP–USP, Brazil — (Permit# 9154/2008 and 13314/2011) and informed written consent was obtained from all participants.

2.2. Sample collection, isolation of peripheral blood mononuclear cells (PBMCs) and RNA extraction

Peripheral blood samples (20 mL) from all participants were collected by standard venipuncture in BD Vacutainer® tubes containing EDTA, followed by isolation of PBMCs by density gradient using Ficoll–Hypaque (Sigma, St. Louis, MO). Total RNA was extracted from the cells using Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol. After that, the purity and the concentration of RNA samples were measured by NanoDrop ND-1000 Spectrophotometer (NanoDrop Products, Wilmington, DE) and their integrity was evaluated by microfluidic electrophoresis using RNA 6000 Nano kit and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples that were protein- and phenol-free and presented RNA integrity number (RIN) ≥ 7.0 were considered for the microarray analysis.

Table 1
Main characteristics of type 1 diabetes mellitus (T1DM) patients and healthy subjects (control group).

	T1DM	Control
Subjects (n)	11	9
Age (years)	23.5 ± 3.9 (18 to 30)	25.1 ± 3.2 (20 to 29)
Gender	3 F/8 M	5 F/4 M
Fasting blood glucose (mg/dL)	134.1 ± 95.8 (23 to 293)	87.4 ± 4.2 (81 to 94)
Glycated hemoglobin (%)	9.5 ± 0.7 (7.2 to 11.1)	–
Duration of diabetes (years)	9.1 ± 4.5 (2 to 16)	–
Insulin therapy	Lanthus/lispro (n = 1) NPH/regular (n = 7) Ultra-fast (n = 1) Lanthus/regular (n = 1) Levemir (n = 1)	–
Metformin (850 mg)	n = 1	–

F, female; M, male; duration of diabetes, period from T1DM diagnosis until the date of enrollment in the present study.

Download English Version:

https://daneshyari.com/en/article/2816515

Download Persian Version:

https://daneshyari.com/article/2816515

Daneshyari.com